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PROTOZOA IN TERMITES OF THE GENUS *AMITERMES*.

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(With Plates XIV and XV, containing Figs. 1–26, and Figs. I–X in the Text.)

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INTRODUCTION.

DURING a collecting trip to Barro Colorado Island, Canal Zone, Panama, in August, 1925, smears were prepared by me from the intestinal contents of *Amitermes beaumonti*, which is abundant in moist decaying wood on the ground in the jungle. In the fall of 1930, during a study of the California species of Amitermes in connection with the field programme of the Termite Investigations Committee, Prof. S. F. Light sent me living material of *Amitermes minimus* Light, *A. emersoni* Light, *A. silvestrianus* Light, *A. coachellae* Light and *A. wheeleri* Desneux.

I am indebted to Prof. Light for the material from California, and to Mr J. E. Zetek for aid in the collection of termites in Panama.

Trichomonas lighti n.sp. (Pl. XIV, figs. 1–10).

A small species of Trichomonas has been found in all species of Amitermes examined for protozoa. It was never encountered in abundance, and in the majority of termites was present in very small numbers. Its occurrence in *Amitermes minimus* is typical of that in the six species studied. Ninety-two individuals of *A. minimus* were examined for Trichomonas, thirty-seven from

one colony and fifty-five from another. In twenty-seven Trichomonas was not seen, indicating that, if not absent, its numbers were very small. In forty-two termites, Trichomonas was recorded as very few or few, the numbers being so small that it was usually necessary to search several fields before finding any. In twenty-three cases the records were given as fairly numerous or numerous, in which case the abundance of Trichomonas was comparable to that of *T. linearis* in *Orthognathotermes wheeleri* (Kirby, 1931). Of ten termites of a third colony of *A. minimus*, which had been kept in the laboratory for a week, seven were negative for protozoa. Trichomonas was fairly numerous or numerous in all specimens of *Amitermes emersoni* and *A. wheeleri* which were examined.

Fixed specimens from *Amitermes minimus* measured 4·5–8 × 3–4·5 microns, and a living specimen 10 × 5·5 microns. Fixed specimens from *Amitermes wheeleri* measured 5–11·5 × 3–7 microns. Some of the largest specimens while living measured 9·5–11 × 5·5–6·5 microns. In some termites the average size of the trichomonads seems to be greater than in others. The flagellates were especially large in those specimens of *A. emersoni* which were examined.

The body is stout and usually rounded anteriorly and posteriorly, with the axostyle projecting from the posterior end instead of laterally, as in some trichomonads.

The four anterior flagella (Figs. 1 and 2) are 8–14 microns long, often being about 10 microns long. In living specimens they either are united into a stout whip, separated at the ends, or completely separated to the base. When they are entirely separate each lashes independently. In some cases one of the anterior flagella is separate from the main group, but in others there is no evidence of a 3 and 1 grouping.

The undulating membrane (Figs. 1 and 2) is similar to that of *Trichomonas labelli* (Kirby, 1931). It is moderately low, has a few bold folds, and there is a rather long free portion of the marginal flagellum.

In all living material studied the axostyle projected for some distance beyond the main portion of the body, but in some fixed specimens there is no projection (Fig. 6). The length of the projecting part frequently is about 2·5–5 microns in fixed material, whereas in living material it is sometimes as long as or longer than the body. In a flagellate 9·5 microns long the posterior projection extended 11 microns beyond the posterior end (Fig. 2).

The axostyle is slender and tapers gradually to the pointed posterior end. Frequently in iron haematoxylin preparations the axostyle is stained (Figs. 4 and 7). That the end of the posterior projection may serve for attachment has been observed in living material from *Amitermes wheeleri*. Several flagellates were observed attached to débris in this manner, with the anterior flagella and undulating membrane beating actively. The body changed position from time to time, but remained attached. Some of the flagellates soon broke away, others remained attached for a long time. The projecting part of the axostyle is probably surrounded by a sheath of cytoplasm. When the posterior projec-

tion extends to a great length (Fig. 2) it is probably the cytoplasm that is drawn out.

A cytostome of characteristic trichomonad form and position is visible in certain preparations (Figs. 1, 5 and 6).

The blepharoplast is a granule of moderate size close to the nuclear membrane. The costa is slender and often cannot be traced to the posterior end in ordinary stained preparations.

The spheroidal or ellipsoidal nucleus is about 2–3 microns in diameter. The granular chromatin is often concentrated beneath the nuclear membrane. In some cases the nuclei, like those of some other trichomonads, appear much like the nuclei of Entamoeba, having granules of peripheral chromatin, a central or somewhat eccentric karyosome-like granule, and some indication of so-called spoke radii.

Although material fixed in osmic vapour and stained with iron haematoxylin, which might be expected to demonstrate the parabasal body, has been studied, that structure has not been observed.

The cytoplasm usually contains many vacuoles enclosing bacteria and other ingested material. This species of Trichomonas appears not to be xylophagous. The cytoplasm often contains, especially in the posterior portion, a number of small spherules which are highly refractile and are stainable with neutral red.

Among the species of Trichomonas previously described from termites, *T. lighti* is similar only to *T. labelli*, which also occurs in one of the Termitidae, *Mirotermes hispaniolae*. It resembles it in almost all features of size and structure, but it seems to differ sufficiently for specific distinction. Its different host distribution presents evidence in favour of its distinction, since there seems to be a restricted host-parasite specificity in the previously described Trichomonadinae of termites, with the exception of *Pentatrichomonoides scroa* (Kirby, 1931). *T. lighti* seems to differ from *T. labelli* in that the costa is not so well developed, the axostyle stains more readily and tapers more gradually to a pointed end, the nucleus is larger and the posterior end usually rounded rather than pointed. Nothing like the projecting lip of the cytostome of *T. labelli* was observed in *T. lighti*.

It was stated in the abstract previously published (1930) that the Trichomonas in *A. beaumonti* is specifically distinct from that in the California species of Amitermes. It was believed at that time that the basis of distinction lay in the stouter costa of the latter, and the stouter axostyle of the former. Further observation has shown that these apparent distinctions do not exist, but were due merely to variations in material and to the technique of preparation. Clearly, it is very difficult to establish definite specific distinctions, based on morphology alone, between such very small flagellates.

Diagnosis. Trichomonas lighti n.sp.

Length 4·5–11·5μ; width 3–6·5μ; four anterior flagella often 8–14μ long; undulating membrane moderately developed, marginal flagellum free for several microns; axostyle fairly well developed, pointed posteriorly, usually

extending some distance beyond the posterior end of the body, often stains with iron haematoxylin; costa slender; nucleus 2–3μ in diameter; parabasal body not observed.

Type host. Amitermes emersoni Light. California.

Additional hosts. Amitermes beaumonti Banks, Panama. *Amitermes coachellae* Light, California. *Amitermes minimus* Light, California. *Amitermes silvestrianus* Light, California. *Amitermes wheeleri* Desneux, California.

Chilomastix minuta n.sp. (Pl. XIV, figs. 11–13).

A small flagellate assignable to the genus *Chilomastix* was found only in *Amitermes beaumonti* of the species of termites investigated during preparation of this paper. In some hosts it is almost as abundant as *Trichomonas lighti*; in others it is infrequent or absent. To my knowledge, this is the first species of Chilomastix to have been recorded from insects.

It is smaller in size than any other species of Chilomastix, the length being only about 6–10 microns, the width 2–4 microns. As it has been found only on stained slides, it has not been possible to determine with absolute certainty the number of free anterior flagella. There are clearly at least two, and in a few cases there was evidence for the existence of the expected number of three.

The identification of this flagellate as Chilomastix derives from the conspicuous cytostomal fibril bordering the left margin of the cytostome, and the position and structure of the nucleus. The fibril extends posteriorly for somewhat less than half the body-length, and is then bent around to the right in the hook-form characteristic of Chilomastix. Sometimes (Fig. 11) it is bent inward at about the middle of the cytostome.

The nucleus is situated as far anteriorly as possible, and is relatively much smaller than in *Trichomonas*. The interior is almost completely filled with a chromatic mass, which sometimes appears granular. The preparations were not favourable for a detailed study of nuclear structure.

The posterior portion of the body is sac-formed and broadly rounded.

The cytoplasm appears coarsely vacuolated, as is characteristic of Chilomastix, and in it are some deeply staining granules and rods, which are probably bacteria. There is no evidence that the flagellate is xylophagous.

In one instance a small rounded form, possibly precystic, was seen (Fig. 13). This was ovoidal, 6 microns long, the nucleus was at the smaller end, and the cytostomal fibril extended most of the length of the body.

Diagnosis. Chilomastix minuta n.sp.

Length 6–10μ; width 2–4μ; fibril around left margin of cytostome deeply staining with iron haematoxylin; nucleus far anterior; body rounded posteriorly.

Host. Amitermes beaumonti Banks, Panama.

Embadomonas termitis n.sp. (Pl. XIV, figs. 14–17).

This, like *Chilomastix minuta*, has been found only in *Amitermes beaumonti*, in some preparations from which it is fairly numerous. Its characters could not be determined with absolute precision from the available material, but it appears to correspond to Embadomonas more closely than to any other described flagellate.

The body is elongated, rounded at both ends, and has a cytostomal depression in the anterior portion. It measures 3–6·5 × 2·5–4·5 microns; average size about 5 × 3·5 microns. There appear to be two flagella, one of which is trailing and somewhat longer than the body. The flagella arise from one or two blepharoplasts located anterolaterally close to the nuclear membrane.

The nucleus, which is located near the anterior end, contains a central karyosome of moderate size and some chromatic granules on the inside of the membrane. It is very different in appearance from the nuclei of Chilomastix and Trichomonas. One binucleate individual was found.

Embadomonas blattae has recently been described from *Blatta orientalis* by Bishop (1931). This species averages seven by six microns, nearly 50 per cent. larger than *E. termitis*, and has a much more conspicuous cytostome. The trailing flagellum is delicate and does not extend much beyond the confines of the cytostome, as it does in *E. termitis*. Except for the number of flagella, the trophozoites and cysts of *E. blattae* bear some resemblance to those of a Chilomastix. The cytostomal fibril is continuous, however. But in *Embadomonas dobelli* from *Rana temporaria*, *Bufo vulgaris* and *Salamandra maculosa*, Miss Bishop described two cytostomal fibrils, only one of which reaches to the posterior end of the cytostome, which is half or more as long as the body. This arrangement of cytostomal fibrils is like that in Chilomastix. In fact, there is, except in the number of flagella, much resemblance between the figures of *E. dobelli* and the figures of *Chilomastix caulleryi* from *Salamandra maculosa* drawn by Grassé (1926). Miss Bishop shows four flagella on some individuals of *E. dobelli* which, she states, were approaching division.

It may seem to the reader that it has not always been possible to distinguish clearly between Chilomastix and flagellates named Embadomonas except by the determination, which is often difficult, of the number of flagella. But there is no doubt in my mind that the forms here named *Chilomastix minuta* and *Embadomonas termitis* represent two separate and well-defined species, even without consideration of the flagella.

Diagnosis. Embadomonas termitis n.sp.

Body stout; length 5 (3–6·5)μ; width 3·5 (2·5–4·5)μ; cytostome large; trailing flagellum longer than body; nucleus anterior, with moderate sized karyosome and peripheral chromatic granules.

Host. Amitermes beaumonti Banks, Panama.

Endamoeba beaumonti sp.nov. (Pl. XV, figs. 19–24).

Amoebae have been observed in *Amitermes beaumonti*, *A. medius*, *A. minimus*, *A. coachellae*, and *A. wheeleri*, but not in thirty specimens of *A. silvestrianus* or thirteen specimens of *A. emersoni*. They were never so abundant as the amoebae in Mirotermes (Kirby, 1927). Of ninety-two individuals of *A. minimus* explored from protozoa, amoebae were found in thirty-five, or 38 per cent.

The species of amoeba appears to be the same in all the hosts. It is similar in size and structure to *Entamoeba thomsoni* Lucas from the cockroach and *Endamoeba disparata* Kirby from *Mirotermes hispaniolae*.

Fixed amoebae from *Amitermes beaumonti* ranged from 11 × 10 microns to 27·5 × 21·5 microns, averaging 17 × 13·5 microns. The largest living amoeba observed, from *Amitermes coachellae*, was 37 microns long and 16 microns broad; other living amoebae measured when rounded up 12–21 microns in diameter.

Activities of the living amoebae have been observed in specimens from *A. coachellae*. Short, broad, lobe-like pseudopodia are formed at various parts of the body by intermittent movements between which the animal is motionless. A clear lobe may be suddenly extruded, and after this burst there may follow an arrest of movement. Endoplasm then flows in quickly, and action is again arrested. When the granules of the endoplasm first enter the clear lobe, active Brownian movement sometimes occurs, soon ceasing. This indicates the temporary low viscosity of the endoplasm when it first enters the lobe. After a pseudopodium forms and is arrested, another sometimes forms close to it, fusing with it; or one is formed in a different region. Sometimes the animal progresses by formation and continuous development of a large, broad pseudopodium at the anterior end of the elongated body.

Attachment of the amoeba by a "foot," as described by Lucas (1927) in *E. thomsoni*, has not been observed in *E. beaumonti*.

The nucleus, which ranges from about 4·5 to 7 microns in diameter, is clearly visible in living material. In some cases it is the first body to enter a forming pseudopodium. It is subject to a certain amount of distortion by pressure of surrounding substances.

The nucleus contains a large central mass of chromatic granules, within which there is often visible a karyosome-like body surrounded by a clear zone. On the inside of the nuclear membrane there also are chromatic granules. These sometimes form a ring in optical section, sometimes there are a few larger granules, sometimes a few large bodies like the peripheral nucleoli of *Endamoeba disparata*. In that species it was shown (Kirby, 1927) that the chromosomes form from the central mass and the nucleoli disappear. In some cases there are visible radial filaments crossing the clear zone between the central mass and the membrane. Brilliant cresyl blue, used dissolved in 1 per cent. solution in absolute alcohol and dried in a film on the slide before placing the amoebae thereon, stains the peripheral chromatic substance deep blue,

while the inner mass remains unstained. After death of the amoebae, if not before, the peripheral nuclear granules stain red with neutral red used in the same way.

Nucleophaga, similar to that in *Endamoeba disparata*, has been observed in a few cases.

The endoplasm contains many refractile granules and bacteria. None of the granules stains with brilliant cresyl blue or neutral red. This amoeba, unlike *E. disparata*, appears not to be xylophagous.

The spindle-formed nuclei which occurred in about half the specimens of *Endamoeba disparata* studied (Kirby, 1927) were not observed in this amoeba.

Entamoeba thomsoni has a central nuclear mass similar to that of this amoeba, but Lucas does not describe large peripheral masses such as those which occur in many specimens of *E. beaumonti*. Also, the activity of the amoeba from termites is quite different from that of the amoeba Lucas described from the cockroach.

Endamoeba beaumonti is the sixth amoeba described from termites, and all of these have been found in the Termitidae. Five were reported (Kirby, 1927) from two species of Mirotermes. *E. beaumonti* has been found in five species of Amitermes.

Diagnosis. Endamoeba beaumonti n.sp.

Length 11–37μ; width 10–21μ; lobe-like pseudopodia formed by expulsive, intermittent movements; nucleus 4·5–7μ in diameter; large, granular, central chromatin mass, with karyosome; scattered chromatic granules or a few large granules beneath nuclear membrane. Feeds on bacteria.

Type host. Amitermes beaumonti Banks, Panama.

Additional hosts. Amitermes coachellae Light, California. *Amitermes minimus* Light, California. *Amitermes wheeleri* Light, California. *Amitermes medius* Banks, Panama.

Nyctotherus silvestrianus n.sp. (Pl. XV, figs. 25 and 26; Figs. I–V).

Dobell (1910) described a species of Nyctotherus from *Kalotermes militaris* of Ceylon as *N. termitis*. Because of the brevity and incompleteness of the description, it is difficult to compare this with other ciliates of the genus. I have found a species of Nyctotherus in Amitermes which resembles *N. termitis* in many ways, but differs from it in size and in details not described in that species. It may be that further study of *N. termitis* would show a close similarity between it and the species from Amitermes, but at present it appears to me necessary to establish a new species. The name *silvestrianus* has been used in recognition of the work of F. Silvestri, who described the genus Amitermes, as well as because it is the specific name of one of the hosts.

Nyctotherus silvestrianus has been found in *Amitermes silvestrianus*, *A. minimus* and *A. emersoni*. In *A. silvestrianus*, eleven of thirty individuals were infected. The number of ciliates in each ranged from one to eleven, averaging five or six. The ciliate was found in members of two of five colonies of *A. mini-*

mus. In one of these, thirty-two of thirty-seven termites were infected. The number of ciliates in each ranged from one to twenty-five, averaging about seven. In some of the hosts cysts were observed. In the case of *A. emersoni*, none of the termites examined from one colony was infected, and only one ciliate was found in one of several termites of another colony. It seems likely that other species of Amitermes would be found to be infected if a sufficient number of colonies were examined.

The length of fifty ciliates from *A. minimus* ranged from 81 to 139 microns, averaging 100 microns; the width from 34 to 86 microns, averaging 62 microns. Five active ciliates were observed 36–60 microns long, 20–36 microns broad; and one was seen 276 × 108 microns. The last was a dividing form. The unusually small ciliates had probably recently emerged from cysts, which are small in comparison with the active forms, measuring 41–48 × 21–26 microns.

Nyctotherus silvestrianus is between *N. termitis* and *N. ovalis* in size. *N. termitis*, according to Dobell (1910), attains a length of 60–70 microns, and a maximum breadth of 40 microns. Bhatia and Gulati (1927) gave the length of *N. ovalis* as 70–350 microns, and Lucas (1927) stated that the cysts of this species measure 55–94 × 25–70 microns.

In form of the body *N. silvestrianus* agrees in general with *N. termitis* and *N. ovalis*. There is some variation in its outline. The posterior end may be broadly rounded (Fig. II), or may taper to a rounded extremity (Fig. I). In some cases there is a concavity on the right side just anterior to the posterior extremity. The flattening of the body is usually greater in the anterior than in the posterior portion. In some cases there is an indentation on the right side at about the level of the nucleus.

The cytopharynx (Figs. 25 and 26; Fig. I), which is fairly straight, begins a short distance anterior to the middle of the body and extends obliquely posteriorly at an angle similar to that followed by the structure in *N. velox* and *N. tipulae*. In *N. ovalis* and *N. termitis* the cytopharynx appears to pursue a more nearly transverse direction. That there is considerable variation in the direction and curvature of the cytopharynx has, however, been shown by Higgins (1929) in *N. cordiformis*. The end of the cytopharynx of *N. silvestrianus* is near the middle of the longitudinal axis of the body, but often it falls short of reaching the middle of the transverse axis.

The cytopyge usually is situated a little to the left of the posterior extremity (Figs. 25 and 26). From it a short canal leads to the contractile vacuole

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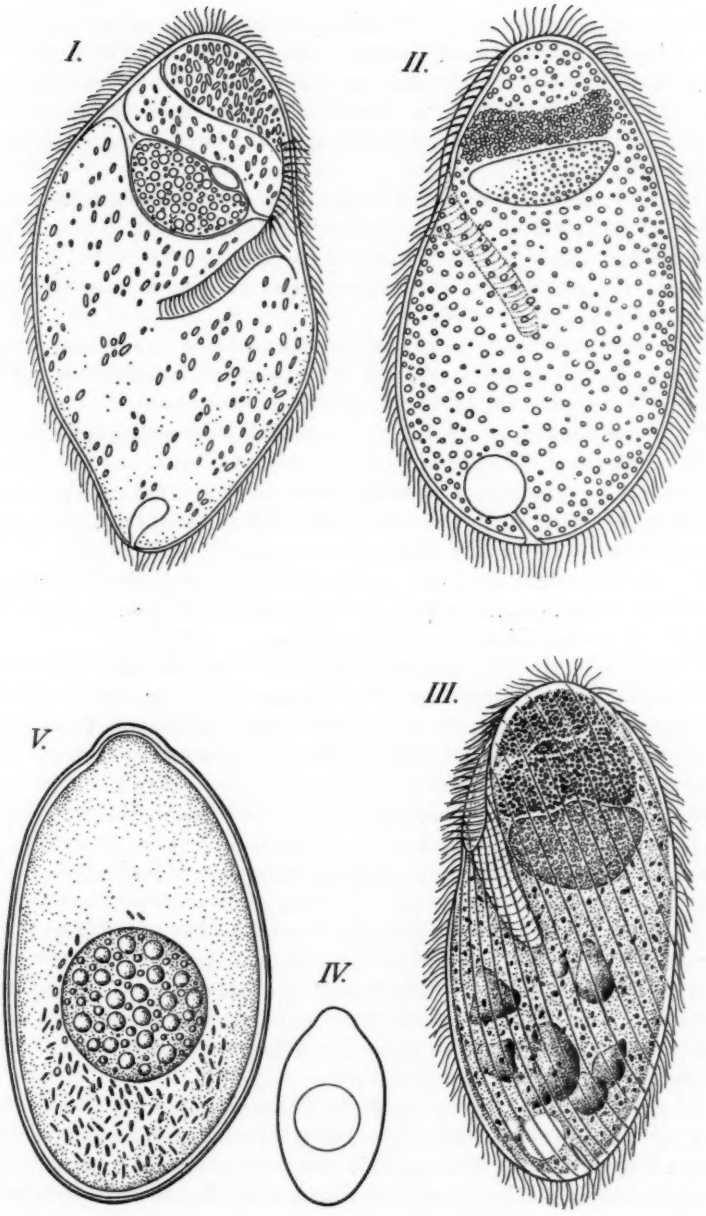
Explanation of Text-figs. I–V

Figs. I–V. *Nyctotherus silvestrianus* n.sp. from *Amitermes minimus* Light. Drawn with camera lucida from unfixed material. I–IV, × 700. I. Stained with neutral red. Only the minute peripheral granules and the macronucleus stained. Figure shows karyophore, frontal lamina, platelets of glycogen-like substance, especially abundant anterior to lamina. II. Glycogen-like platelets concentrated between nucleus and frontal lamina. III. Platelets of glycogen-like substance stained with iodine. Figure shows ciliary lines and groups of parasitic bacteria. IV. Outline of cyst drawn at same magnification as trophozoites. V. Detail of cyst, spherical macronucleus and platelets of glycogen-like substance. × 1780.


I.
II.
III.
IV.
V.

(Fig. II). In one case the interval between the pulsations of the vacuole was three minutes.

The macronucleus in most individuals is elongated transversely, rounded at its ends, and more flattened anteriorly than posteriorly. The macronuclei of this form in eighteen specimens measured 8–36 × 24–48 microns, averaging about 18 × 35 microns. In small ciliates, however, the macronuclei are broadly ellipsoidal, approaching the spherical form of the nuclei in the cysts (Fig. V).

The macronucleus is surrounded by membranes which are extended toward the periphery of the body as a karyophore (Fig. I; fig. 25). In its possession of a well-developed karyophore, *N. silvestrianus* agrees with *N. velox*, *N. ovalis*, *N. termitis* and other members of the sub-genus *Nyctotherus* s.str. established by Grassé (1928).

The macronucleus contains spherules and granules of various sizes, ranging in diameter up to 1·5 microns (Fig. V). It stains red with neutral red and blue with cresyl blue, while the micronucleus remains unstained.

The micronucleus is situated on the anterior surface of the macronucleus, usually somewhat to the right of its centre (Fig. 26; Fig. I).

After reaching the edge of the body, one membrane of the right part of the karyophore is turned upward and backward to the left, to form what Grassé called the frontal lamina in *N. haranti* from a gecko (Fig. 26; Fig. I). This extends to the left margin of the body, and is situated about halfway between the macronucleus and the anterior end. Dobell's figure of *N. termitis* shows what is apparently a frontal lamina in that species.

From the end of the cytopharynx there extends a slender, band-formed structure which stains deeply with iron haematoxylin (Figs. 25 and 26). This is as long as or longer than the cytopharynx. It is not attached at its distal end, as its position in the body varies and it may be curled or looped in various ways.

This structure clearly is homologous with the "continuation tube" of the "subpharyngeal canal" described by Higgins (1929) in *N. cordiformis*. Higgins described a continuous canal from the surface of the body to the end of the cytopharynx along its right side, and on through the "continuation tube." Near the end of the pharynx she found that the tube widens into an ampulla. The subpharyngeal tube and ampulla may stain deeply. In *N. silvestrianus* the filament connects directly with the end of the cytopharynx, and there is no evidence that it is merely an extension of a structure which lies beside the cytopharynx. No structure resembling the "subpharyngeal canal" described by Higgins has been observed.

Lying against the cytopharynx of *N. silvestrianus* is a peculiar structure which has the form of a V whose right limb is the stouter (Fig. 25). The bend is situated a short distance from the end of the cytopharynx on its upper side. At the bend there is a deeply staining enlargement (Fig. 26). The stouter limb extends along the wall of the cytopharynx near but to one side of the right

margin, so that it does not occupy the position of Higgins' subpharyngeal canal in *N. cordiformis*. It has been traced for about two-thirds of the distance to the cytostome, where it becomes attenuated. Its substance is heterogeneous, consisting of alternating stainable and non-stainable elements (Fig. 25).

The other limb is much more slender. It is situated along the dorsal side of the cytopharynx near but a short distance from the bases of the pharyngeal membranelles. It has been traced as far as the cytostome. Arising from this limb of the V near the bend are several deeply staining fibrils which extend into the endoplasm, where, so far as could be determined from the material, they end freely. For several microns beyond their point of origin they extend outward; then they bend sharply posteriorly, forming a group of fibres which can be traced farther for a few microns.

Along the margin of the cytopharynx close to the bases of the membranelles there appears to be a slender, stainable fibril which rounds the end and perhaps meets the enlargement at the apex of the V.

The writer has been able to give only an incomplete account of the structures as they appeared to him in a few specimens of *N. silvestrianus* from *Amitermes silvestrianus* fixed in Schaudinn's fluid and stained with iron haematoxylin. Probably a study of more adequate material prepared by various methods including sectioning would disclose more minute details. The structures are suggestive of certain parts of the neuromotor systems described in various ciliates.

Ten Kate (1927) showed near the end of the cytopharynx of *N. ovalis* an "ectoplasmic thickening" suggestive of the basal part of the V-shaped structure described above. The structure which he designated as the pharynx apparently corresponds to the proximal part of the stainable filament in *N. silvestrianus*. It has not been possible for the writer to determine whether or not this is an open tube continuous with the cavity of the cytopharynx. It stains deeply where attached, but in other portions its edges stain more deeply than the interior. Ten Kate showed many fibrils extending from near the end of the cytopharynx, which he named prepharyngeal fibrils. Possibly some of these correspond to the fibrils mentioned above in *N. silvestrianus*. Similar structures were shown by Entz (1913) in *Nyctotherus piscicola*.

The filament extending from the end of the cytopharynx of *Nyctotherus* resembles the band-formed pellicular fibre described by Lynch (1930) at the end of the cytopharynx of *Lechriopyla mystax*. The V-shaped structure may have some relation to the structure described by Lynch as the neuromotorium of that ciliate from the sea urchin.

The endoplasm of the living animal contains many ellipsoidal, spindle-shaped or disc-formed granules which stain brown with iodine (Figs. I–III). These were referred to by Grassé (1928) as platelets of glycogen. They are often closely packed in the region anterior to the nucleus, forming the granular area (Körnerfeld) in *N. ovalis* and other species (Stein, 1867). The granular region anterior to the nucleus is divided by the frontal lamina. Individuals vary in

the degree of concentration of the glycogen-like material here. In some there is a dense mass anterior to the lamina, in some posterior to it, and in some in both parts of the prenuclear region. The platelets are dissolved in material fixed in Schaudinn's fluid, leaving an alveolar cytoplasm.

There are many minute granules, located especially in the peripheral region, which stain with neutral red and cresyl blue.

In several specimens aggregations of minute, haematoxylin-staining granules were abundant throughout the postnuclear cytoplasm (Figs. 25 and 26). These appear to be the same as the clusters of granules described by Sassuchin (1928) as parasitic bacteria in *Nyctotherus ovalis*.

The cysts are like those of *N. ovalis*, having at one end a thickened protuberance (Figs. IV and V). The body of the encysted ciliate contains a relatively large, spherical macronucleus and an aggregation of the glycogen-like bodies, described by Lucas (1928) in *N. ovalis* as spindle-formed bodies.

Diagnosis. Nyctotherus silvestrianus n.sp.

Length 100 (36–139)μ; width 62 (20–86)μ; cysts 41–48 × 21–26μ, shaped like the cysts of *N. ovalis*; cytopharynx extending obliquely posteriorly, ending near middle of longitudinal axis; macronucleus 35 (24–48)μ × 18 (8–36)μ in larger specimens, spheroidal in cysts and newly excysted specimens; micronucleus on anterior surface; karyophore and frontal lamina present; long band-formed filament extending from end of cytopharynx; V-shaped structure, from which arise some filaments, close to cytopharynx; small platelets of glycogen-like material abundant, often concentrated anterior to nucleus; small peripheral neutral red staining granules.

Type host. Amitermes silvestrianus Light, California.

Additional hosts. Amitermes minimus Light, California. *Amitermes emersoni* Light, California.

Coccidian (Figs. VI–X).

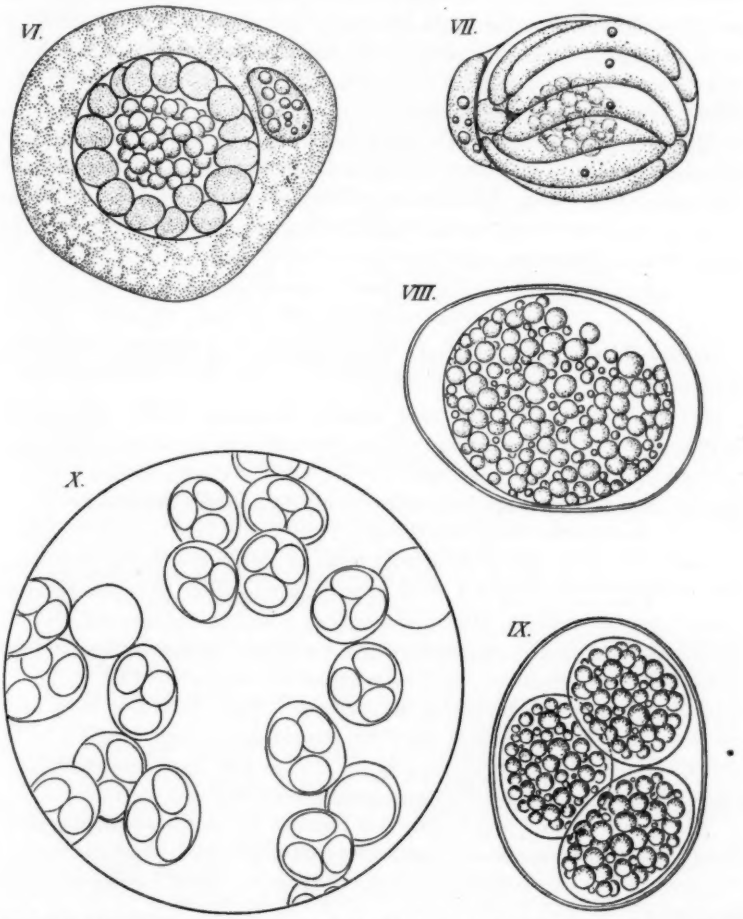
A coccidian was found in only one individual of *Amitermes minimus*, although fifty-five other termites of the same colony were examined, as well as many from other colonies of this and other species. A considerable number of oocysts were present in the intestine. Within the tissues of a fragment of the intestine three instances of schizogony, some macrogametocytes and some oocysts were seen.

In the stages of schizogony which were observed the schizonts had given rise to sixteen banana-formed merozoites each about 15 microns long, leaving a rather large residual body (Fig. VI). Seen in optical section (Fig. VII) the merozoites were circular and about 3 microns in diameter. These groups of merozoites were enclosed in cells, each containing a nucleus.

In the lumen of the intestine, as well as in certain of the cells, oocysts were numerous. After remaining in 2 per cent. potassium bichromate for four days the enclosed body had divided into three equal parts (Figs. IX and X). As there were three equal parts in all of the many cases observed in which sporula-

tion had occurred, it is probable that this number is always produced. No further sporulation occurred. Probably development was not completed.

The oocysts are broadly ellipsoidal, measuring about 21–28 × 18–22



Figs. VI–X. Coccidian from *Amitermes minimus* Light. 1–4, ×1830. VI. Optical cross-section of sixteen merozoites and residual body resulting from schizogony within a nucleated cell. VII. Similar stage from side view. VIII. Oocyst, with contents undivided. IX. Oocyst, with contents divided into three sporoplasts with sporocysts. X. A field of the microscope, showing numerous oocysts most of which have sporulated to the stage shown in Fig. IX. ×530.

microns. The wall is rather thick and perfectly smooth, and sometimes has a brownish tinge. The sporocysts are also broadly ellipsoidal, measuring about 11–13 × 8·5–9·5 microns. No residual body is left in the process of sporulation.

In the absence of observations on the type of microgametocyte activity and

microgamete formation, or of the sporozoites, it is not possible to give a systematic assignment to the coccidian. In some cases there appeared to be evidence of the association of gametocytes characteristic of the Adeleidea. The formation of three sporoblasts suggests the same condition in Chagasella. The oocyst wall of Chagasella is, as shown in the plates of Chagas (1910), much more delicate than in the coccidian from Amitermes; though in the text Chagas states that a thick membrane is formed. Otherwise, all stages observed could belong to a species of Chagasella.

This is the first record of a coccidian from a termite, and the third of a sporozoan other than gregarines. Perez (1908) reports the microsporidian *Duboscquia legeri* from *Reticulitermes lucifugus*, and Georgévitch (1930) mentions having found a species of Plistophora in the Malpighian tubules of the same species of termite.

Other micro-organisms (Pl. XV, fig. 18).

In contrast to the relatively small numbers of protozoa, other micro-organisms are very abundant.

In *Amitermes beaumonti* very slender filaments, which vary greatly in length, occur in large numbers. Some are only 1 or 2 microns long, some 40 microns, but many are more moderate in length. Among them are some very slender, closely coiled spirals, up to 10 microns long. Probably some of the filaments are spirals straightened out.

Very numerous is a short spiral of one or two turns, tapered at the ends, and in many cases about 1 or 2 microns long. There is often a chromatic granule in the middle, sometimes others elsewhere. Duboscq and Grassé (1927) reported similar organisms from *Kalotermes* (*Glyptotermes*) *iridipennis*, and noted the resemblance to *Paraspirillum vejdovskii* Dobell.

A few larger, stouter spiral forms occur. One, with few turns, was 17 microns long. This resembles the form named *Spirochaeta termitis* by Dobell (1910). Some of the more slender spirals of moderate length resemble the form called *Treponema hilli* by Duboscq and Grassé (1927).

Abundant are rod-like forms, 2–8 microns in length, straight or curved, deeply staining or containing some chromatin granules. Some of these were observed in transverse division. Some of them resemble *Fusiformis termitidis* Hoelling.

In the five species of Amitermes from California similar micro-organisms are equally abundant.

Summary.

1. Six species of Amitermes, one from Panama and five from California, have been examined for protozoa. Certain protozoa have been found in many or all individuals examined of all species, but they are much less abundant than are flagellates in termites of the three xylophagous families below the Termitidae.

2. *Trichomonas lighti* n.sp. has been found in all species, but not in all individuals.

3. *Chilomastix minuta* n.sp. occurs in *Amitermes beaumonti*.

4. *Embadomonas termitis* n.sp. is described from *Amitermes beaumonti*.

5. *Endamoeba beaumonti* n.sp. has been observed in five host species. In its size and nuclear structure it resembles *Endamoeba disparata* of *Mirotermes hispaniolae*.

6. *Nyctotherus silvestrianus* n.sp. has been found in three of the Californian species. It resembles *N. termitis* and *N. ovalis*, but is larger than the former and smaller than the latter. A long filament is attached to the end of the cytopharynx. A thickened, **V**-shaped structure lies against the cytopharynx. Several deeply staining fibrils arise from this **V**.

7. A coccidian has been found in one individual of *Amitermes minimus*. This forms sixteen banana-formed merozoites and three sporoblasts as in Chagasella.

8. Micro-organisms other than protozoa are very abundant in the intestines of all six species of Amitermes, and they are similar in all.

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EXPLANATION OF PLATES XIV AND XV.

Abbreviations for methods of preparation: H., Heidenhain's haematoxylin; Os., osmic vapour; S., Schaudinn's fluid; R., Regaud's haematoxylin.

PLATE XIV.

All figures × 1830.

Figs. 1–10. *Trichomonas lighti* n.sp.

Fig. 1. Composite diagram.

Fig. 2. From *Amitermes wheeleri*, drawn from a living flagellate.

Fig. 3. From *A. minimus*. A specimen of unusually small size. Not all flagella shown. Os., H.

Figs. 4–5. From *A. beaumonti*. S., H.

Figs. 6–10. From *A. emersoni*. Os., S., R.

Fig. 6. Shows cytostome.

Fig. 7. Stained axostyle.

Fig. 8. Unstained axostyle.

Figs. 11–14. *Chilomastix minuta* n.sp. from *A. beaumonti*. S., H. Showing cytostomal fibril. There are probably three anterior flagella, though the figures show less than that number.

Figs. 15–17. *Embadomonas termitis* n.sp. from *A. beaumonti*. S., H.

PLATE XV.

Fig. 18. Various forms of micro-organisms from *Amitermes beaumonti*. S., H. × 1830.

Figs. 19–24. *Endamoeba beaumonti* n.sp. × 1830.

Figs. 19–23. From *A. beaumonti*. S., H.

Fig. 23. *Nucleophaga* parasitic in nucleus.

Fig. 24. From *A. minimus*.

Figs. 25–26. *Nyctotherus silvestrianus* n.sp. from *A. silvestrianus*. S., H. × 970.

Fig. 25. Macronucleus; karyophore; V-shaped structure against cytopharynx, with filaments arising from near apex; band-shaped filament at end of cytopharynx; parasitic bacteria.

Fig. 26. Same structures, micronucleus and frontal lamina.

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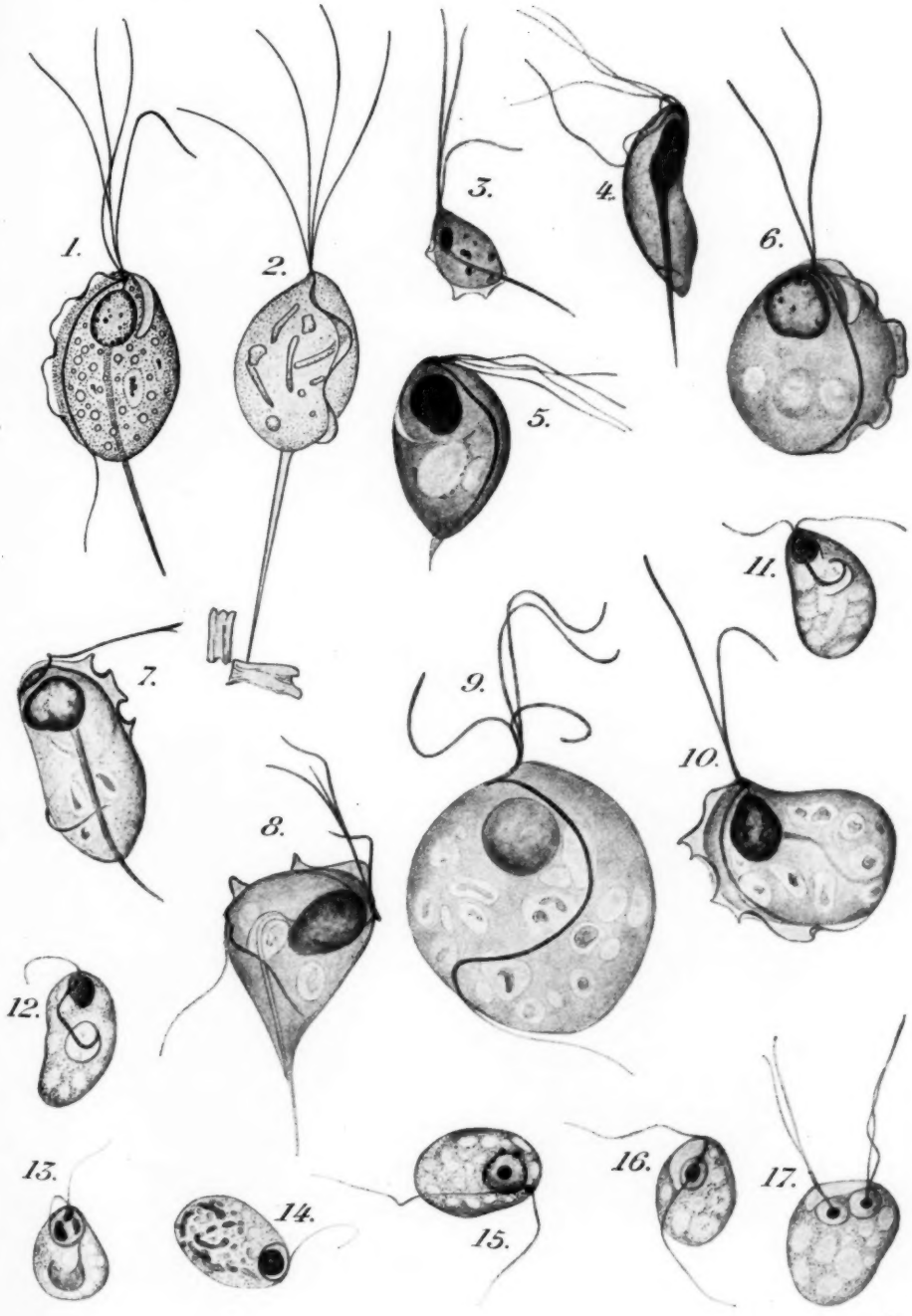
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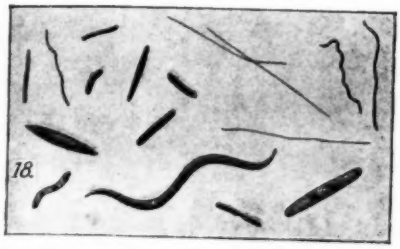
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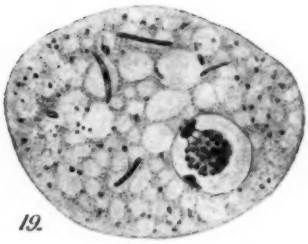
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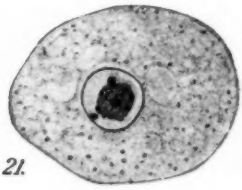
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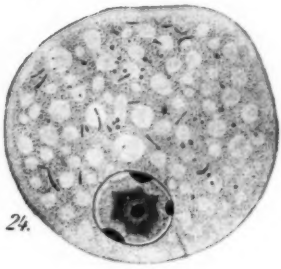
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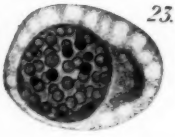
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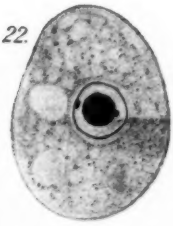
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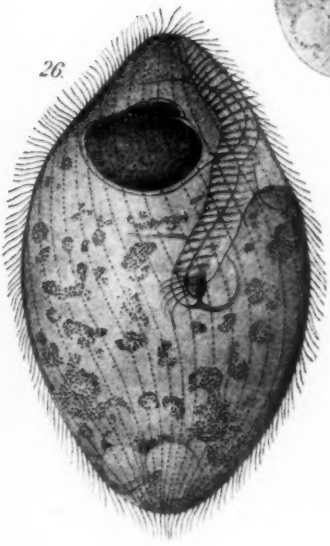
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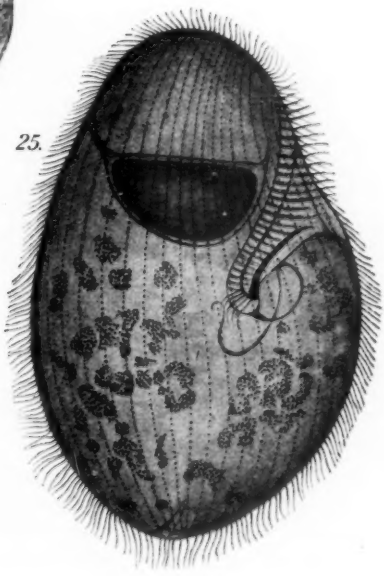
25.

A RAPID METHOD OF STAINING AND MOUNTING MID-GUTS OF MOSQUITOES FOR THE DEMONSTRATION OF OOCYSTS.

By RICHARD GREEN, M.D.

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It is often desirable that a mid-gut showing oocysts be preserved for demonstration purposes or for record. The method herein described seems to have certain advantages over those usually recommended in saving considerable time during fixation, staining and mounting of the specimen, also in permitting pigment within oocysts being seen on any part of the mid-gut. The stain used (toluidine blue) is not taken up to any great extent by the nuclei of the gut cells, but appears to have some degree of selective action on the younger oocysts.

When a series of mid-guts is being examined, the following routine procedure for the preservation of those found to contain oocysts has been adopted by the writer:

(i) 1 per cent. formaldehyde in normal saline is run under the coverslip and the excess of fluid removed with small strips of blotting paper until a suitable degree of flattening is obtained. The slide is then put aside for about 10 minutes in a closed Petri dish containing moist blotting paper.

(ii) Pugh's stain[1] is then run under the coverslip and, if the staining of the specimen is watched under a dissecting microscope, the mid-gut will be seen to have adhered, as a rule, to the slide or the coverslip, usually the latter. The stain is allowed to act for about five minutes.

(ii) The coverslip is gently floated to one side and removed. The specimen

[1] Pugh's stain is made up as follows: Dissolve 1 g. of toluidine blue in 20 c.c. absolute alcohol. Add this solution, together with 20 c.c. glacial acetic acid, to 1000 c.c. distilled water. (Pugh's stain is generally used for the staining of *C. diphtheriae*. The toluidine blue has a special affinity for the metachromatic granules within the organism.)

"Formalin" usually contains 36 to 38 per cent. formaldehyde. To make a solution containing 1 per cent. formaldehyde and 0·85 per cent. sodium chloride, one volume of "formalin" is taken and made up to 36 or 38 volumes by adding distilled water. To every 100 c.c. of this solution is added 0·85 g. sodium chloride.

Prior to the preliminary fixation, the oocysts may be manœuvred to the edge of the mid-gut, if desired, by withdrawing some of the normal saline (in which the gut has been dissected out and placed for inspection) and rolling the mid-gut by sliding the coverslip. The "formol-saline" is then added and, during withdrawal of the excess of this fluid, the degree of flattening of the gut is controlled.

It is essential that the slides and coverslips used be free from greasy material.

(on the coverslip or slide) is then dipped carefully into the following mixtures which are kept in closed jars:

(*a*)	Acetone, 95 parts:	Xylol 5 parts,	for	30 seconds
(*b*)	Acetone, 70 „ :	Xylol 30 „		1 minute
(*c*)	Acetone, 30 „ :	Xylol 70 „		1½ minutes
(*d*)		Xylol „		2 minutes

The specimen is then put, facing upwards, under a dissecting microscope and excess of xylol removed with blotting paper.

(iv) When the last remnant of xylol is evaporating from round the mid-gut, a drop of clove oil is placed on the specimen. The excess of clove oil is removed as before and a suitable quantity of Canada balsam or Euparal is put on. A coverslip or slide, as the case may be, is then lowered on to the mounting medium and the specimen is examined under the dissecting microscope. Pressure is then applied and maintained on the coverslip, the mid-gut being flattened to the desired degree, until the mounting medium has "set" sufficiently. Further clearing takes place during the next hour or so, at the end of which time the mid-gut is ready for examination under the ordinary microscope and may be regarded as a "permanent" preparation.

Figures and photo-micrographs showing the appearance of an infected mid-gut which has been stained and mounted by the process described above will be found in my paper: A malarial parasite of Malayan monkeys and its development in anophelene mosquitoes, *Trans. Roy. Soc. Tropical Med. and Hygiene*, **25**, 455–477, May 1932.

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ON SOME BRITISH LARVAL TREMATODES FROM TERRESTRIAL HOSTS.

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(With 13 Figures in the Text.)

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I. Introduction.

In this paper I have described five species of Scottish larval trematodes from terrestrial molluscs. Four of these appear to be new and for them I propose the names, *Cercaria politae alliariae, C. politae nitidulae, C. politae lucidae* and *C. lauriae cylindraceae*, corresponding to the names of the molluscan hosts from which they were obtained. The other species, *Cercariaeum helicis* Braun from *Helix hortensis* Müll. is also described in detail. The larvae were found in the course of a systematic search of the land invertebrata of the Dundee and Arbroath districts for larval helminth parasites and were investigated by methods similar to those given in a former paper (Harper, 1929).

II. Description of parasites.

Cercariaeum helicis Braun (Figs. 1 and 2).

The sporocysts were obtained from the liver of *Helix hortensis* Müll. They are elongated, much branched and exceedingly involved. Lengths from 1 to 3·5 mm. are common, the breadth being about 0·14 mm. The colour at maturity is orange-yellow and in the older specimens numerous black pigment spots appear in the outer layer of the wall. The latter is tough and shows numerous transverse constrictions. Young sporocysts are slightly motile, but the power

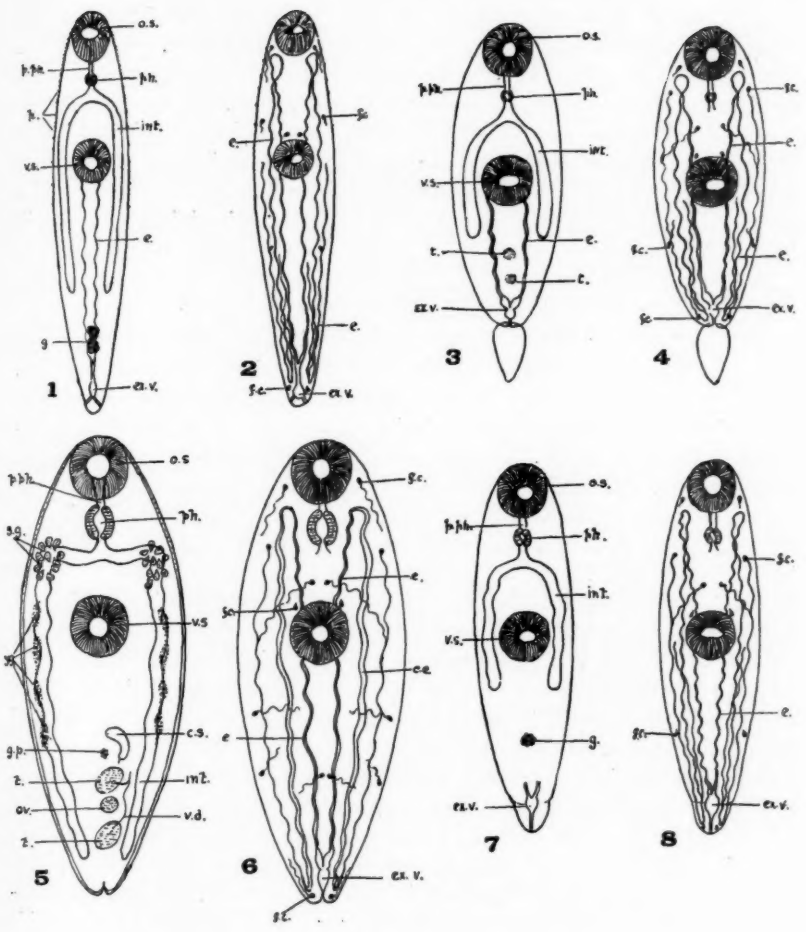
of independent movement is soon lost. From 20 to 30 actively moving cercariae may be present within one sporocyst. The germinal area is non-localised and there is no birth-pore. The liver and gonads of the infected mollusc are usually quite destroyed.

The cercaria is straw-coloured and muscular, moving actively in a leech-like manner by means of two powerful suckers. Its length is approximately 0·46 mm. and the maximum breadth 0·07 mm., about one-third the distance from the anterior end. The oral sucker measures 0·045 mm. across; it is directed ventrally and is unarmed. A ventral sucker of diameter 0·04 mm. is situated in the anterior third of the body. Anteriorly, also, the body bears numerous minute papillae best seen at the lateral margins. There is no tail.

Internal anatomy. A short tubular prepharynx leads to an oval muscular pharynx. The latter leads almost directly to a thin-walled triclad intestine the loops of which reach about two-thirds the length of the body. A pear-shaped contractile excretory vesicle lies at the hind end and connects with the postero-lateral margins of the body by means of two narrow tubules. Anteriorly the vesicle gives off a short median collecting canal which soon bifurcates. Each lateral canal so formed loops at the level of the lower margin of the oral sucker to form a secondary tubule which in turn loops at the posterior end of the body. The tertiary tubule thus formed was traced anteriorly to the level of the ventral sucker. The secondary excretory receives a tributary near its posterior end. Ciliary motion was observed anteriorly at the junction of the primary and secondary tubules. Flame cells occur in the positions indicated in Fig. 2, but their capillaries were not fully traced. The genital system is represented by an imperfectly divided mass of small cells in the posterior third of the body.

Identification. From the description above and from observations made on the advanced larval stage of this cercaria obtained from the same host species, there seems little doubt that it is *Cercariaeum helicis* Braun. References in the literature, however, deal more fully with the advanced larval stages of the species than with the sporocyst and cercaria.

The earliest reference appears to be that of Meckel (1846) who described a narrow tailless sexually immature distome 0·77 mm. long from the *garden snail.* The excretory system in his larva is on the same basis as in the cercaria described above. Braun (1891) described the same larva from *Helix pomatia* L., *H. nemoralis* L. and *H. arbustorum* L. and named it *Cercariaeum helicis.* The advanced larval stage of the latter has an oval mouth sucker and a somewhat smaller ventral sucker at the hinder limit of the anterior third of the body. The pharynx lies just behind the oral sucker and the oesophagus forks immediately behind it, the intestinal loops extending along the sides of the body to almost the posterior margin. Testes and ovary lie in a straight line in the posterior third of the body while the genital pore is in the median line near the end of the middle third. Blochmann (1892) found the cercaria particularly in *Helix hortensis* Müll., and claimed that it reaches sexual maturity in the hedgehog *Erinaceus europaeus*, the adult being *Distomum caudatum* v. Linst. (= *D. lepto-*



GROUP A.

Figs. 1 and 2. *Cercariaeum helicis* Braun (1) showing alimentary canal, etc., (2) excretory system.

Figs. 3–6. *Cercaria politae alliariae* n.sp. (3) showing alimentary canal, etc. (4) excretory system. (5) Advanced larval stage showing alimentary canal and glands, (6) excretory system of the same.

Figs. 7 and 8. (7) *Cercaria politae nitidulae* n.sp. showing alimentary canal, etc. (8) excretory system.

Lettering: *c.e.* ciliated canal of excretory system; *c.s.* cirrus sac; *e.* excretory vessel; *ex. p.* excretory pore; *ex. v.* excretory vesicle; *f.c.* flame cell; *g.* rudiment of genital system; *g.p.* genital pore; *int.* intestine; *oes.* oesophagus; *o.s.* oral sucker; *ov.* ovary; *p.* papilla; *ph.* pharynx; *p. ph.* prepharynx; *s.g.* salivary gland cell; *st.* stylet; *t.* testes; *ut.* uterus; *v.d.* vas deferens; *v.s.* ventral sucker; *y.g.* yolk glands.

stomum Olsson). *Cercariaeum helicis* Braun was used by Bettendorf (1897) as a source for his paper on the musculature and sense organs of Trematodes. Hofmann (1899) found a big infection (95–96 per cent.) in *H. arbustorum* L. and described the sporocysts as much branched structures, the young cercariae as white in colour and the older ones as brownish yellow. The unarmed cuticle bears anteriorly numerous *Tastwarzchen* which become less conspicuous in the older larvae. The genital and excretory systems are described by him in some detail. Roewer (1906) found definitely formed young distomes of this species together with all stages (not described) from the miracidium onwards in *H. arbustorum*.

Cercaria politae alliariae n.sp. (Figs. 3–6).

The sporocysts of this species occur in the liver of *Polita alliaria* Miller. They are long greyish white structures, often reaching a length of 1·5 mm., with a maximum breadth of 0·15 mm. Movement is slight, and appears to be due mainly to the active cercariae within. The area of cell proliferation is non-localised and from 20 to 30 actively moving cercariae may be present within one sporocyst. No birth-pore was observed.

The cercaria is transparent and exceedingly muscular. Its outline is oval with a length of 0·25 mm. and a maximum breadth of 0·1 mm. The oral sucker is directed ventrally, and the ventral sucker is situated near the middle of the body. A short stumpy tail is present in all the younger cercariae, but is shed before or very soon after they leave the sporocyst.

Internal anatomy. An oval muscular pharynx is preceded by a tubular prepharynx and followed by a very short oesophagus which divides to form two club-shaped loops of intestine reaching a short distance below the level of the ventral sucker. The excretory vesicle is Y-shaped, the median stem only being contractile. The excretory pore is terminal. From each cornu of the vesicle a narrow collecting canal passes anteriorly to loop at the level of the lower margin of the oral sucker. The secondary canal so formed loops near the posterior end of the larva then passes forward to end in two capillaries, each with terminal flame cells in the anterior third of the body. The secondary excretory canal receives a tributary about the level of the excretory vesicle. This tributary was traced anteriorly to the level of the lower margin of the ventral sucker and in that distance it remains unbranched. In all, six flame cells were observed on each side of the body. All the tubules and capillaries of the excretory system are much convoluted and their relative positions vary greatly with the amount of extension and contraction of the body. The genital system is represented by two testes placed one behind the other in the posterior third of the body. For other measurements see Table I.

Activity and duration of free life. The duration of free life appears to be longest when the cercariae are kept on blades of damp grass. On growing grass moistened by frequent sprayings with tap water, cercariae were observed creeping on the blades after a period of 24 hours. On dry grass they do not get

sufficient moisture to prevent desiccation and soon die. The larvae are negatively phototropic. In tap water they survive from 15 to 20 hours.

Table I.

	mm.
Oral sucker	0·048
Ventral sucker	0·048
Length of tail	0·052
Breadth of tail at base	0·04

On the advanced larval stage of Cercaria politae alliariae *n.sp.* (Figs. 5 and 6).

Along with sporocysts of this species I have found, on several occasions, numerous larval forms from the cercaria stage onwards, in the liver and gonads, between the body wall and the shell, and in the mantle cavity of the host. Specimens up to 1·5 mm. in length were obtained. The most marked development observable in the larger forms is an increase in the number of excretory tubules and flame cells, and a more complete differentiation of the genital system. I have also found these larval stages, but in smaller numbers, in the molluscs *Polita alliaria* Miller and *P. nitidula* Drap. not infected with sporocysts, thus indicating that the cercaria is capable of migrating from the first host.

External features. The larva being exceedingly contractile, varies greatly in shape. When first isolated from the host it is commonly broadly oval or almost spherical in outline with the cuticle thrown into numerous transverse convolutions. Very soon it becomes actively mobile so that accurate measurement is difficult. The largest observed measured approximately 1·45 mm. long with a breadth of 0·66 mm. near the centre of the body. The oral sucker is subterminal and measures 0·17 mm. in diameter. The ventral sucker is slightly smaller, measuring 0·15 mm. across and is situated near the junction of the anterior and middle thirds of the body. The body is straw-coloured and relatively smooth, but there are indications that the adult will have an armature of spines anteriorly. These spines were observed through the cuticle in the older specimens, but only in a few were they seen above the surface. They are most abundant in the extreme anterior region and thin off posteriorly, disappearing about the level of the ventral sucker.

Internal anatomy. The prepharynx is short and the pharynx muscular measuring 0·08 × 0·06 mm. The intestine bifurcates almost immediately behind the pharynx and the intestinal loops pass out at right angles, then bend abruptly and follow a sinuous course to near the posterior end of the body. Food particles derived from the liver and gonads of the host are usually abundant in the loops.

A group of flask-shaped cells with granular contents are situated around the shoulders of the intestinal loops. No ducts were observed leading from these glands and it seems probable that they connect directly with the intestine. Hofmann (1899) recorded glands in a similar situation in *Cercariaeum helicis* Braun.

The excretory vesicle is composed of two anterior non-contractile lobes and a median contractile stem on the surface of which are prominent longitudinal muscular striations. The excretory pore is terminal. The primary collecting tubule entering each lobe is relatively narrow and contains numerous rod-shaped excretory granules in its lower portion. The secondary excretory tubules are richly ciliated, long patches of cilia alternating with shorter non-ciliated portions. The disposition of the main accessory tubules remains the same as in the cercaria, but many finer tubules may now be observed forming a complicated network throughout the body. These finer tubules take origin mainly from the tertiary excretory tubules and their tributaries and from the tributaries of the secondary excretory tubules. Two additional flame cells were observed on each side of the body.

Two testes are readily observable as clear oval bodies lying in the posterior third of the body. They measure 0·075 × 0·065 mm. The two vasa deferentia unite to form a single tube a short distance behind a wide cirrus sac. The ovary lies between the testes and has a diameter of 0·58 mm. A portion of the convoluted uterus was observed in the middle third of the body. The vitellaria occur on each side of the body in its middle third. The genital aperture is situated in the middle line about 0·48 mm. from the posterior extremity.

Identification. I have found no reference in the literature to a cercaria similar to the above. The structure of its advanced larval stage bears some resemblance to *Cercariaeum spinosulum* (Hofmann, 1899) but the sporocysts of the latter species are brown in colour, measure as much as 7 mm. long, and occur chiefly in *Helix arbustorum* L., a very much larger mollusc than *Polita alliaria* Miller. The internal anatomy of the advanced larval stage of the species described here is also somewhat similar to that of *Cercariaeum helicis* Braun but in it there are no anterior spines.

Cercaria politae nitidulae n.sp. (Figs. 7 and 8).

The sporocysts which were obtained from *Polita nitidula* Drap. are long, transparent, sparingly branched sacs varying in length from 1 to 1·7 mm. Normally there are but one or two branches on each sporocyst and a constriction is frequently present at their junction with the parent. The diameter, 0·13–0·16 mm., is fairly uniform. The free ends of the sporocysts oscillate slowly from side to side. The area of cell proliferation is non-localised, lining the whole cavity of the sporocyst. From 25 to 30 actively moving cercariae may be present in a sporocyst at one time. No mature cercariae were seen in any of the branches. No birth-pore was observed.

The cercaria is transparent and tapers towards the posterior end, the maximum breadth being just anterior to the ventral sucker. The larva is exceedingly muscular, assuming at times an elongated and at others an almost globular appearance when the cuticle throughout the whole length of the body is thrown into numerous transverse corrugations. There are two suckers, an oval sucker round the mouth and an imperforate ventral sucker situated near

the centre of the body. The ventral sucker protrudes markedly when the larva elongates and its position varies with the degree of extension and contraction. There is a slight constriction on the body about 0·028 mm. from the posterior end but there is no tail.

Internal anatomy. The oral cavity is followed by a short tubular prepharynx and a prominent muscular oval pharynx. There is practically no oesophagus and the loops of the intestine reach a little below the level of the ventral sucker.

The contractile excretory vesicle is roughly spherical and leads almost directly to two primary collecting tubules. The excretory pore is terminal. The arrangement of the other excretory tubules and the position of the flame cells resembles that of *Cercaria politae alliariae* (p. 310). The genital system is only feebly differentiated, consisting of a spherical mass of small round cells situated in the median line about mid-way between the ventral sucker and the posterior end of the body.

Other measurements of the cercaria are given in Table II.

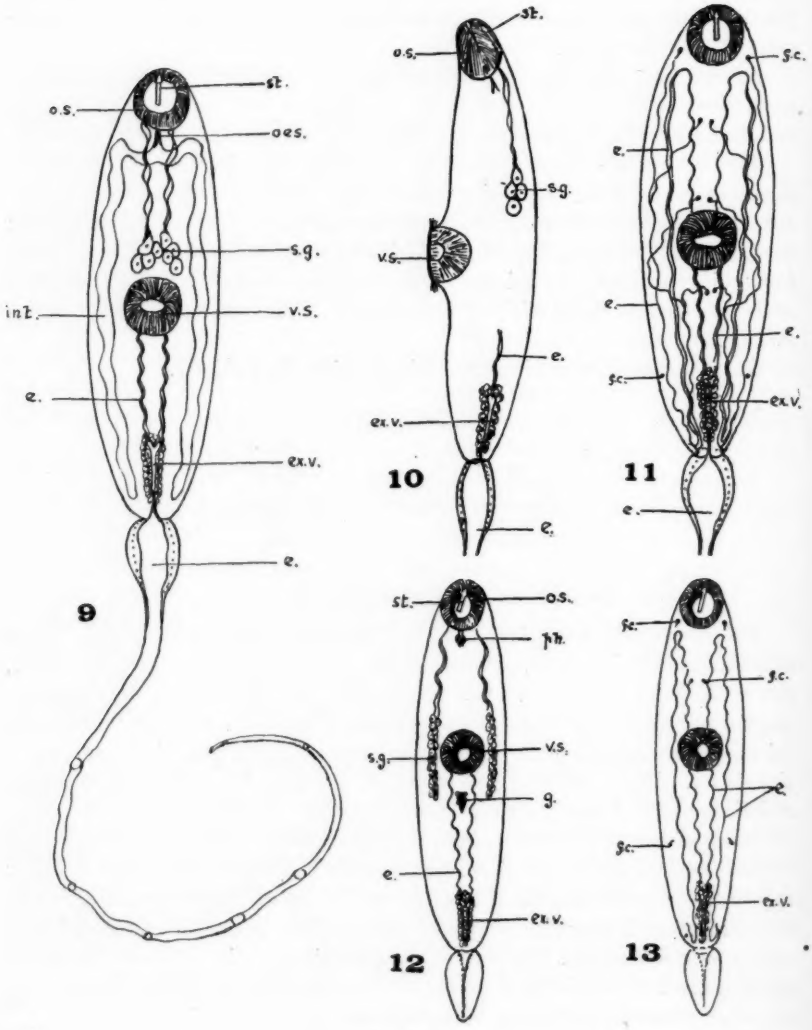
Table II.

	mm.
Length	0·27–0·32
Breadth anteriorly	0·08
Breadth posteriorly	0·055
Oral sucker	0·04
Ventral sucker	0·04

Cercaria politae lucidae n.sp. (Figs. 9–11).

The sporocysts were found in the liver of *Polita lucida* Drap. When mature, they are elongated, unbranched sacs with an average length of 3·5 mm., and a breadth of 0·33 mm. They are greyish white in colour with numerous small black pigment dots in the outer layers of the wall. The wall increases in thickness towards the poles of which an anterior and a posterior may be distinguished. At the anterior pole there are prominent longitudinal muscular striations and a distinct vertical slit-like opening extending through the whole thickness of the wall in that region. In uncompressed specimens the aperture of this terminal slit was at times seen to open and close slowly. This opening appears to be of the nature of a birth-pore for the escape of cercariae, and to a certain extent it may also be adhesive in function. It was not observed in the very young sporocysts. The wall at the posterior pole is markedly thickened and it is towards this end that germ ball formation is most active. As many as 30 active cercariae may occur in one sporocyst.

The cercaria is almost cylindrical and fairly transparent. The anterior half of the body is specially contractile. The resting length is about 0·33 mm., but it may stretch to twice that length when the larva is in motion. The cuticle is firm and thick and contains numerous refractile granules arranged in an irregular network. Similar granules are also present in the proximal portion of the tail. The oral sucker is directed ventrally and is armed with a stylet 0·23 mm. in total length. The distal end of the stylet is reinforced by two



GROUP B.

Figs. 9–11. *Cercaria politae lucidae* n.sp. (9) showing alimentary canal and glands, (10) lateral view, (11) excretory system.

Figs. 12 and 13. *Cercaria politae cylindraceae* n.sp. (12) glands, etc., (13) excretory system.

For lettering see Figs. 1–8 (p. 309).

lateral blades directed forwards and downwards, and a sharp dorsal ridge. The ventral sucker is situated near the middle of the body and protrudes slightly. Its outer margin bears a row of short bristles best seen when the cercaria is viewed laterally. The tail is more transparent than the rest of the larva and measures fully twice the length of the body. It is for the most part slender but a contractile bulbous swelling occurs at its junction with the body, and at intervals along its length is a varying number (3–7) of round vacuole-like structures. The latter stand out bead-like and at times appear more and at other times less dilated.

Internal anatomy. A short prepharynx leads to a feebly developed pharynx which is almost contiguous with a relatively broad thin-walled intestine. The diverticula pass out at right angles and, after a short distance, bend abruptly backwards to extend along the sides of the body to the posterior end. It is not possible to define accurately the limits of the intestine in the active uncompressed cercaria. They show up best when the larva is examined in normal saline under the weight of a cover glass until active movement has almost ceased.

The excretory vesicle is bluntly bilobed and elongated in the median line. It is dorsal in position and slowly contractile. The wall is lined with a layer of large flattened cells with prominent nuclei, while entirely surrounding the vesicle is a glandular mass composed of round cells containing yellowish brown granules. Each lobe of the vesicle receives a main lateral collecting tubule which passes towards the head region where it loops and passes back to the posterior end where it loops again, the tertiary tubule so formed passing anteriorly to a flame cell at the postero-lateral margin of the oral sucker. The secondary excretory tubule is considerably wider than the others. In all, 12 flame cells were observed, 6 on each side of the body, but the capillaries associated with the posterior pair were not traced. The distal end of the excretory vesicle leads to a single median tubule running the whole length of the tail. The caudal tubule often contains numerous excretory granules. An excretory pore was not observed.

An imperfectly divided glandular mass, dorsal in position and composed of 8 cells with finely granular contents and prominent nuclei lies a short distance anterior to the ventral sucker. Two convoluted ducts pass forward from the mass to open in the roof of the oral cavity. In their course the ducts exhibit a number of dilatations. Other measurements of the cercaria are given in Table III.

Table III.

	mm.
Breadth of body	0·09
Oral sucker	0·04
Ventral sucker	0·042
Length of tail	0·5–0·7
Breadth of tail at base	0·05

Activity and duration of free life. When the cercaria is placed in water the tail coils and uncoils itself slowly. It is, however, unable to swim, but may survive 20–24 hours in tap water. On damp grass it progresses in a leech-like

fashion, aided only very slightly by the tail which is readily cast. In such a habitat it may survive for 30 hours.

The presence of a tail in this cercaria is curious. Vaney and Conte (1899) recorded a similar feature in *Cercaria pomatiae* n.sp. and these authors consider that the tail, which they often found shed in the sporocyst, probably plays no part in the dissemination of the species. Linstow (1898) has also recorded a long tailed cercaria from *Zebrina detrita*, and *Cercaria ericitorum* n.sp. a shorter tailed species from *Xerophilus ericetorum* Müll., which, under ordinary circumstances, had no opportunity of getting into water.

Cercaria lauriae cylindraceae n.sp. (Figs. 12 and 13).

The sporocysts are short, colourless, spindle-shaped sacs developing in the liver of *Lauria cylindracea* Da Costa. When mature, they measure 0·77–1 mm. in length, with a breadth of 0·18–0·22 mm. They are not numerous in any one infected mollusc. Young sporocysts are transparent and slightly motile; at maturity they are almost inert. The wall is thin and easily ruptured. The germinal epithelium lines the whole cavity and usually there are 10–12 active cercariae in a sporocyst at one time. No birth-pore was observed.

The cercaria is translucent and of an elongated oval outline. The average length is 0·27 mm. and the maximum breadth 0·055 mm., about one-third the distance from the anterior extremity. The oral sucker is oval and armed in its roof with a slender stylet 0·02 mm. in length. The ventral sucker is situated near the centre of the body; its margin is crenated and tinged with brown pigment. The tail is stumpy and a longitudinal fissure is constant on its ventral surface.

Internal anatomy. A small spherical pharynx lies behind the oral sucker but no other portion of the intestine could be made out.

The excretory vesicle is thick-walled and elongated in the median line. Ventral to it lies a cluster of round cells with dark brown granular contents. The anterior end of the vesicle is bluntly bilobed and from each lobe a narrow lateral tubule passes anteriorly to loop at the lower margin of the oral sucker. The secondary excretory tubule was traced to the level of the excretory vesicle and in that distance it remains unbranched. Flame cells were observed in the positions indicated. The excretory pore opens dorsally at the posterior end of the vesicle.

An elongated glandular mass composed of numerous small oval cells containing finely granular contents occurs on either side of the ventral sucker. From each glandular mass a sinuous duct passes forwards to open in the roof of the oral sucker.

The genital system is represented by a group of small round cells, situated dorsally and slightly posterior to the level of the ventral sucker.

For other measurements of the cercaria see Table IV.

Table IV.

	mm.
Oral sucker	0·035
Ventral sucker	0·03
Length of tail	0·05
Breadth of tail at base	0·03

III. SUMMARY.

Five larval trematodes have been obtained from a search of many terrestrial molluscs. All are distome cercariae developing in sporocysts. One species appears to be *Cercariaeum helicis* Braun the sporocyst and cercaria stages of which are described here in detail. The other four species are new. *Cercaria politae lucidae* n.sp. and *C. lauriae cylindraceae* n.sp. the two Xiphidiocercariae described are rare in this locality, each species being found only on two occasions. The larvae obtained are also less abundant and less representative of the larval Trematode groups than those met with during my search of the local fresh-water molluscs for similar parasites (Harper, 1929, 1931).

During the investigation two new records have been added to the molluscan fauna of Forfarshire (Vice county, No. 90), viz. *Polita lucida* Drap., and *Succinea elegans* Risso. No helminth parasites were found in the last-named mollusc.

IV. ACKNOWLEDGMENTS.

I wish to record my indebtedness to the late Prof. Gemmill, F.R.S., under whom this work was carried out during my tenure of a Research Maintenance Allowance from the Department of Scientific and Industrial Research (1923–5) and a Carnegie Research Scholarship (1925–6). I have also to thank Mr H. A. Coates, F.S.A. (Scot.) of Perth, through whose kindness the molluscan hosts were named and Prof. D. Rutherford Dow, M.D., in whose department this paper was prepared.

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ON A NEW SPECIES OF TREMATODE (*PROHEMISTOMUM SERPENTUM* N.SP.) FROM A SNAKE, WITH A NOTE ON AN IMMATURE SPECIES OF *HETERECHINOSTOMUM* ODHNER FROM THE CAT.

By B. S. GOGATE.

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(With 2 Figures in the Text.)

Prohemistomum serpentum n.sp. (Figs. 1 and 2.)

Description. Body more or less elongated, narrowing anteriorly and posteriorly. Length 1·34–1·44[1], maximum width 0·476, maximum thickness 0·433, both measured in the region of adhesive apparatus. Entire surface of the body, except adhesive apparatus and the region posterior to second testis, covered with fine spines. Ventral folds originate approximately at centre of body and meet a little behind the level of posterior third to form a shallow cavity occupied by the adhesive apparatus. Adhesive apparatus smooth, oval, 0·4–0·44 long, 0·26–0·3 wide, with narrow central depression, anteriorly reaching acetabulum and posteriorly anterior margin of posterior testis. In it are imbedded vitelline follicles, anterior testis, ovary and anterior portions of cirrus sac and uterus.

Oral sucker terminal, 0·103–0·126 long and 0·064–0·08 wide. Pharynx globular, 0·046 long and 0·053 broad, immediately following oral sucker. Oesophagus narrow, 0·106 long. Intestinal caeca only traceable up to commencement of adhesive apparatus. Acetabulum 0·064 in diameter, weakly developed, clearly seen only in sections, in whole mounts masked by vitelline follicles.

Testes large, smooth, one behind the other, on one side of centre line of body: anterior testis almost circular 0·166 in diameter: posterior testis a little smaller, 0·153 long and 0·166 broad, lying half-way across posterior limits of ventral folds. Cirrus sac 0·353–0·473 long, with internal vesicula seminalis and cirrus, ventral to testes and partly covered by uterus.

Ovary ovoid, 0·14 long and 0·12 broad, dorsal to testes, with anterior portion covered ventrally by anterior testis. Vitelline follicles obscure Laurer's canal. Vitellaria well developed, composed of large follicles 0·08–0·094 long and 0·06–0·066 wide filling adhesive apparatus except for the space occupied by the genital organs previously mentioned, the central narrow depression and a very

[1] All measurements in mm.

narrow area surrounding it. Uterus ventral to cirrus sac. Genital pore terminal, surrounded by circular muscle fibres. Genital sinus absent. Egg large, thin shelled, 0·12–0·14 long and 0·073–0·08 wide.

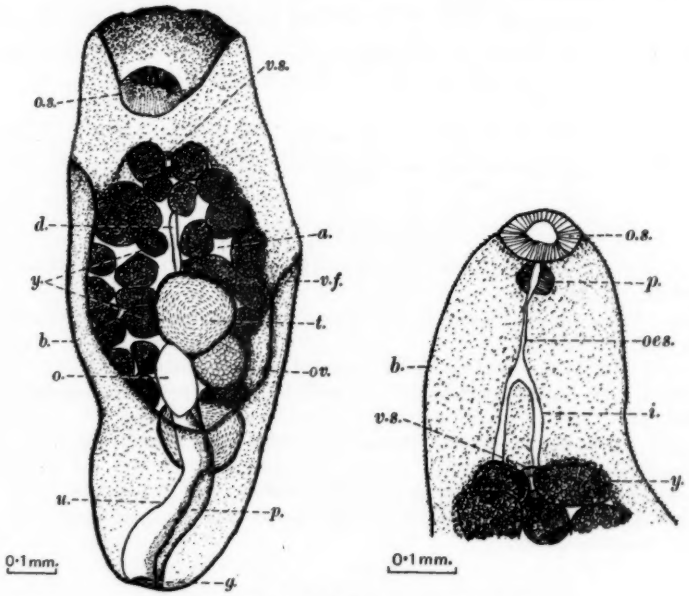
Host. Natrix piscator Schneider 1799.

Location. Intestine.

Locality. Rangoon.

This species differs from the others of the same genus as follows:

(1) The large size of the adhesive apparatus. *P. industrium* Tubangui 1922 has this organ so well developed that it extends to the oral sucker and this is



Figs. 1 and 2. *Prohemistomum serpentum* n.sp.

Fig. 1. Entire worm: *a.* adhesive apparatus—*b.* body spines—*d.* central depression—*g.* genital pore—*o.* egg—*o.s.* oral sucker—*ov.* ovary—*p.* cirrus pouch—*t.* testis—*u.* uterus—*v.f.* ventral folds—*v.s.* ventral sucker—*y.* yolk glands.

Fig. 2. Anterior part of worm: *i.* intestine—*oes.* oesophagus—*p.* pharynx.

clearly distinct. Excluding this species, the nearest is *P. appendiculatum* Ciurea 1916, in which the apparatus is 0·15–0·245 long and 0·125–0·200 broad, and is clearly separated from ventral sucker.

(2) Vitellaria confined to adhesive apparatus. The only other species possessing this character is *P. industrium* Tubangui 1922.

(3) The limitation of uterine eggs to 1–2. The nearest number to this is in *P. appendiculatum* Ciurea 1916, and *P. spinulosum* Odhner 1913, where 4–5 eggs are to be found.

(4) Minor differences in size and position of various organs. I therefore regard it as a new species, which I name *Prohemistomum serpentum*.

Heterechinostomum magnovatum Stunkard and Haviland 1925.

Some immature specimens were obtained from the intestine of a domestic cat. All structures were well developed except the cirrus sac and the uterus; the former being rudimentary and the latter completely undeveloped.

The specimens differ from all other species in the possession of 24 collar spines instead of 20–22 as given by Odhner (1910) in the generic diagnosis.

In other characters they closely resemble *Heterechinostomum magnovatum* Stunkard and Haviland 1925 from the rat and, in the absence of mature specimens, are provisionally assigned to that species. The generic diagnosis should therefore be altered to "20–24 collar spines."

ACKNOWLEDGMENT: I wish to express my indebtedness to Dr F. J. Meggitt for placing at my disposal the material described in this paper.

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SOME LARVAL TREMATODES FROM THE COAST IN THE REGION OF ROSCOFF, FINISTÈRE[1]

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(With 13 Figures in the Text.)

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Introduction.

Examination of larval trematodes at Roscoff was begun in an attempt to find there two species previously reported by Lebour (1912) at Plymouth, England. One of them, *Cercaria lophocerca* Lebour, is probably not identical with *C. lophocerca* Filippi, but may be identical with a cercaria shown experimentally by Stunkard (1930 *a*) to be a larval stage of *Cryptocotyle lingua*. The other species, *C. purpurae*, according to Lebour (1914) and Lebour and Elmhirst (1922), is the larval stage of *Parorchis acanthus* Nicoll. It is very similar to a species described by Stunkard and Shaw (1931) as *C. sensifera* and experi-

[1] The observations herein recorded were made at the Station Biologique, Roscoff, during August, 1931. To Prof. Ch. Pérez, Director of the Station, I am indebted not only for the laboratory facilities, graciously extended, but also for his interest in the investigation. The notes were prepared for publication during the winter of 1931–2, while I was enjoying the hospitality of the Institut für Schiffs- und Tropenkrankheiten, Hamburg, through the kindness of its Director, Prof. Dr F. Fülleborn. The work here reported is incidental to other studies on the life-history of parasitic flatworms which I was pursuing during the year as Fellow of the Guggenheim Foundation, New York.

mentally demonstrated by Cable and Stunkard (1931) to be a larval stage of *Parorchis avitus* Linton. Nicoll has suggested that the American and European species are identical, although Linton regarded them as distinct. The discovery of the larval stages of the European forms would permit a comparison with those of the American representatives and solve the question of specific identity. Unfortunately, although over ten thousand snails were examined, including large numbers of those which harbour the species described by Miss Lebour, neither of them was obtained.

This paper makes no pretence of being a survey of the larval trematodes of the region. No historical treatment is attempted, and comparisons with described forms are largely omitted since the descriptions of earlier authors are often too imperfect and incomplete to permit a positive identification of the species with which they worked. Consequently, specific determination of some of the material at hand is not at all certain.

Material and methods.

Large numbers of snails were collected in the Roscoff district. They were arranged according to species and maintained in small aquaria. From ten to one hundred snails, depending on the size of the molluscs, were placed in each dish. At the end of each 12 hours the snails were transferred to other dishes containing fresh sea water. The water in which they had been kept was then examined for cercariae. The method is not new; in fact it was recommended by Pagenstecher (1862). In the study of marine cercariae, where the incidence of infection is slight and where many snails must be examined, the method affords many advantages. Especially, it provides a constant supply of living cercariae for study. The method has a distinct disadvantage, however, since one does not know which of the perhaps one hundred small snails in a given container is infected, and in case the infected snail dies before the study of the cercaria is completed, knowledge of the stages which occur within the snail is lacking. This difficulty may be avoided by placing each snail in a separate dish for 12 to 24 hours to determine which carries the infection.

Specific description of larval trematodes is beset with many difficulties. Indeed, in his excellent monograph, Sewell (1922) raised the question, "whether cercariae can be distinguished specifically by a study of their structural anatomy." He stated: "I am of the opinion that in the vast majority of cases definite specific recognition is possible only when our knowledge of the structure of the cercaria and of its parthenita is complete to the minutest detail, and even so there are certain cases, such as that of the cercaria of *Schistosomum japonicum* and *Cercaria Indica XXX*, in which it seems impossible to arrive at a final decision except through a series of breeding experiments conducted in order to obtain the final adult stages. A further difficulty... is the fact that in the vast majority of cases the records and descriptions of the various cercariae discovered by early workers are totally inadequate: roughly speaking, no

description published before the year 1914 is sufficiently adequate to enable one with certainty to recognise and identify any cercaria recorded prior to that date." He maintained further that, "we are by no means certain that many of the cercariae, that have been so meagrely described by earlier writers and yet have been given specific names by them, are actually true species, and I am moreover entirely opposed to the principle of conferring specific names on larval forms."

An analysis of the methods used in the study of larval trematodes was given by Stunkard (1930 *b*). It was there pointed out that cercariae obtained by the crushing of snails are frequently immature, and since a considerable proportion of existing descriptions were made from such cercariae, the accounts may be confusing. Even accurate descriptions may be misleading unless the methods used in the study are fully stated. The size of specimens varies enormously with different treatment. The tendency of various fixing and preserving fluids to shrink or swell specimens is well known and needs only passing mention. There is considerable initial difference in size of normally emerged living cercariae from the same snail. The larvae manifest much difference of form as elongation and retraction occur, and measurements of the same specimen vary with the amount of pressure exerted by the cover-glass. With a thin cover-glass and an abundance of water the pressure may be negligible, while evaporation of water may increase the pressure until measurements of length and breadth are twice as great as corresponding measurements of the same specimen when able to contract without pressure. Each specific description should be accompanied therefore by an adequate statement of the methods employed.

Since experimental demonstration of trematode life histories is so difficult and slow, progress must be made to a large extent by the accumulated knowledge of these organisms acquired bit by bit through the observations of different workers. Accordingly, some method must be adopted to designate particular forms. While there may be valid objections to giving them specific names, there are obvious corresponding advantages, and although a given form may be shown ultimately not to be a true species, the rules of nomenclature define proper procedure in any case. Descriptions of the larvae are justified, even if positive identification cannot be made, and incomplete descriptions if accurately and carefully done afford important contributions to knowledge and may aid other workers in the solution of life-history problems. Frequently the structure, behaviour, and distribution of cercariae afford clues to later stages in the life cycle.

The study of metacercariae is of special importance, since specimens in this stage often retain cercarial features that relate them to particular cercariae, and at the same time they may be sufficiently developed to indicate the final adult form, which of course reaches maturity in another host.

Accordingly, various invertebrates, notably worms and crustaceans, were examined for the presence of encysted metacercariae. These invertebrate hosts

are incapable of extended migrations; they tend to remain in the same area, and consequently the cercarial and adult stages of their trematode parasites are presumably to be found in the immediate vicinity.

Seven species of cercariae and four species of metacercariae were studied.

Previously described cercariae.

Cercaria rhodometopa Pérez 1924.

(Figs. 1 and 2.)

This species was characterised briefly and named by Pérez (1924). Further study has confirmed his observations and permits a more extended description. The location of the parasites in the genital gland of *Turritella communis* Risso and their effect upon the host was reported by Pérez. Sporocysts may be present in enormous numbers and almost entirely fill the terminal portion of the snail's body. When freed in sea water, the sporocysts are curved or slightly coiled structures, 2–3 mm. long and 0·4–0·6 mm. wide. They are bright orange, due to the presence of pigment in their walls. Sections show the wall to be composed of two layers, an outer one containing large numbers of deeply staining nuclei closely packed together, and an inner one which contains few, faintly staining nuclei. Cell boundaries are not distinct. The sporocysts are filled with cercariae in various stages of development.

The cercariae (Fig. 1) have large, thin bodies and long, powerful tails. In swimming the body is much contracted, bent ventrally, and the tail lashes vigorously. The tail has lateral membranous fin-folds, strengthened by cuticular rays. In one specimen, thirty-six of these rays were counted in the anterior two-thirds of the tail, while other smaller ones were present farther back. The fin-folds make the tail a very effective swimming organ. When in contact with firm surfaces, the larvae attach themselves by their suckers and manifest creeping movements. The acetabulum is situated slightly behind the middle of the body and measures 0·06–0·08 mm. in diameter.

The body of the larva is 0·4–0·9 mm. long by 0·2–0·48 mm. wide. The tail is 0·57–1·2 mm. long and when extended about 0·05 mm. wide at the base. When retracted it is about twice as wide. The most conspicuous feature of the

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The drawings to Figs. 1–13 are freehand composite sketches, made from numerous observations and sometimes completed from the study of serial sections.

Explanation of Text-figs. 1–4*a*.

Fig. 1. *Cercaria rhodometopa*; entire specimen, slightly compressed.

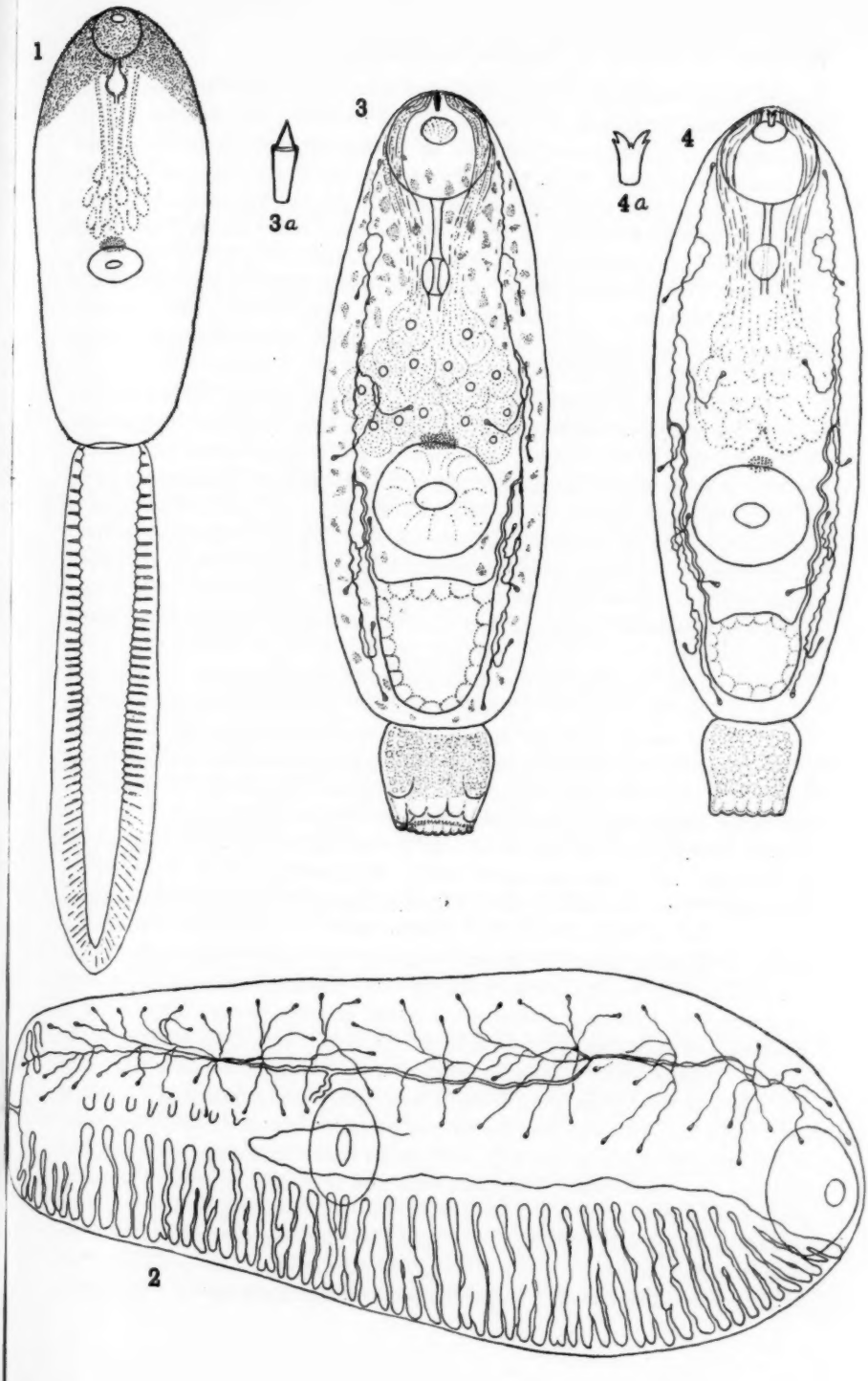
Fig. 2. *Cercaria rhodometopa*; excretory system. The vesicle and its evaginations are represented only on the right side of the drawing while the flame cells and capillaries are shown for only the left side.

Fig. 3. *Cercaria linearis*; entire specimen, slightly compressed.

Fig. 3 *a*. *Cercaria linearis*; stylet.

Fig. 4. *Cercaria brachyura*; entire specimen, slightly compressed.

Fig. 4 *a*. *Cercaria brachyura*; stylet.


1
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3a
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cercaria is the rose-pink colour of the anterior end. The colour is faint at first, becoming more intense as the cercaria attains maturity. The cuticula is thick and spined. The body-wall contains the usual circular, longitudinal, and diagonal muscle fibres. In the parenchyma there are gland cells of at least two different types. One kind, scattered irregularly through the body stains uniformly with haematoxylin. In front of the acetabulum there is a cluster of gland cells which stains *in vitro* with neutral-red, and after fixation with eosin. These cells are numerous, lobed, and consequently difficult to count. Their ducts pass forward to open at the anterior end of the body. In form and position they correspond to the penetration glands of other cercariae, and presumably these cells have a similar function.

The digestive and reproductive systems are only slightly developed. The oral sucker measures 0·08–0·094 mm. in diameter. There is a short prepharynx and a pharynx, 0·026–0·03 mm. in diameter. The commissure of the nervous system is clearly visible behind and above the pharynx, but no other portions of the alimentary tract could be distinguished, either in living cercariae or in fixed and sectioned material. The reproductive system is represented by a cluster of deeply staining cells, situated immediately in front of the acetabulum. These cells are undoubtedly the anlagen of the terminal portions of the genital ducts and copulatory organs. The beginnings of the gonads themselves could not be located.

The excretory system has a complicated and interesting form. I regret that it is not possible to give a full description of this system, but as only one crushed snail was available for study, observations were limited to a single day. With additional material it should be possible easily to complete the observations, *i.e.* to determine precisely the manner of branching in the system and in the flame cell groups. I was unable to trace the excretory system in the tail, and all observations are concerned with the portion in the body. The vesicle is Y-shaped and bears numerous lateral evaginations, many of which are branched. There are three or four lateral evaginations from the bladder and seven to nine on each side of the common median stem of the vesicle. The lateral diverticula from the anterior crura occupy much of the body in this region. The vesicle opens at the caudal tip of the body and anteriorly it extends to the region of the oral sucker. It is dorsal in position and the manner of branching is shown in the right side of Fig. 2 (p. 325). This portion of the system contains many refractive, concretionary bodies. On either side, near the region of the acetabulum, a duct arises from the vesicle and after a short sinuous course, divides into anterior and posterior branches. Each branch is again subdivided, giving rise ultimately to six flame cell groups, each of which contains five flame cells. The flame cells and ducts are ventral in position, sixty on each side of the body, as shown in the left side of Fig. 2. The excretory system is apparently well developed and probably has already attained the definitive form which will persist through all succeeding stages in the life cycle of the species. The system is so peculiar and characteristic that it will afford a

quick and certain criterion for the identification of later stages in the life history, and may, indeed, lead to the correlation of this larva with an adult trematode having the same excretory pattern.

Cotylocercous cercariae.

This group of short-tailed cercariae was separated by Dollfus (1914) from the microcercous cercariae, since the tail in these larvae acts as an adhesive organ. Other morphological and biological characteristics were listed by Dollfus, and although his definition of the group requires revision, there appears to be abundant evidence for regarding these forms as distinct.

The first representatives of the group were described by Lespés (1857) as *C. brachyura* and *C. linearis*. His descriptions are brief, his figures not distinctive, and recognition of the larvae is correspondingly difficult. Both species develop in simple sporocysts, *C. brachyura* in *Trochus cynereus* and *C. linearis* in *Littorina littorea*. Both have stylets, that of *C. brachyura* is small with two short lateral projections, whereas the stylet of *C. linearis* is larger and according to the figure, simple in form. *C. brachyura* measured 0·2 mm. in length, while *C. linearis* is more elongate. According to Lespés, *C. brachyura* has two salivary glands and a single duct on each side of the body, while *C. linearis* has four such cells in all, each with its own duct. The tail of *C. linearis* is smaller. In the figures the bodies of the two species are about the same size, that of *C. linearis* is slightly longer and narrower. The tail of *C. brachyura* is figured as between one-fourth and one-third of the body-length, whereas that of *C. linearis* is one-seventh of the body-length.

The next year Diesing (1858) renamed *C. brachyura*, calling it *C. pachycerca*. He had previously (1850) given the name *C. brachyura* to a cercaria from *Planorbis submarginata* described by Filippi as *Distomum polymorphum*. The renaming of *C. brachyura* Lespés 1857, would be justified only if it were cogeneric with *C. brachyura* Diesing 1850. Since "Cercaria" was treated formerly as a generic name, the change appeared to be justified and *C. pachycerca* has been used as the name of this species by several later authors. But "Cercaria" is the name of a super-generic group; *C. brachyura* Lespés is certainly not cogeneric with *C. brachyura* Diesing, and consequently the name of Lespés must be restored.

Pagenstecher (1862) described the third member of the group as *C. cotylura* from *Trochus cinereus* at Cette. He noted the characteristic movements of the larvae, creeping by the successive attachment of opposite ends of the body, and swimming by an eel-like or serpentine wriggling. This cercaria measures 0·33–1 mm. long, the acetabulum is larger than the oral sucker and no stylet is present. The tail is about one-eighth of the body-length.

The next report is that of Pelseneer (1906) who described what he called *C. pachycerca* Lespés from *Trochus cinereus* at Wimereux. He noted that the sporocysts were orange-brown (once almost colourless), and that the young ones are rediaform. The acetabulum of the cercaria is larger than the oral

sucker, there is no mention of a stylet, and the digestive system bifurcates shortly behind the pharynx, long cylindrical caeca extending to the level of the acetabulum. Encystment in *Trochus* was reported.

Lebour (1912) redescribed *C. linearis* Lespés, *C. brachyura* Lespés, and reported a new species, *C. buccini* from *Buccinum undatum*. *C. linearis*, according to Lebour, develops in inert, brilliant orange sporocysts which occupy the kidney and other organs of *Littorina littorea*. The cercaria measured only 0·18 mm. in length, including the tail; tail one-eleventh of body-length. The oral sucker is 0·035 mm. in diameter and slightly smaller than the acetabulum. The stylet is cleft anteriorly. The stylet glands are conspicuous in front of the acetabulum and four ducts pass forward to open beside the stylet. Lebour described *C. brachyura* as developing in active, orange-coloured sporocysts in the digestive gland of *Gibbula* (= *Trochus*) *cinerea*. The cercaria is 0·24 mm. long without the tail; the body is spined; the oral sucker measures 0·05 mm. in diameter and is slightly larger than the acetabulum. There is no stylet, but four ducts lead forward from stylet glands. *C. buccini* occurs in the digestive gland of *Buccinum undatum*. This species develops in pale yellow sporocysts. The cercaria is 0·33 mm. long, including the tail. The oral sucker and acetabulum measure 0·045 mm. in diameter, the stylet has four points directed anteriorly; the median pair are longer and more pointed than the outer ones. The stylet glands are situated between the suckers and three ducts pass forward on each side of the body.

Sewell (1922) stated that "The whole subject of the microcercous cercariae is at present in a state of extreme confusion." From the cotylocercous cercariae he separated a smaller "Linearis" group in which he included *C. pachycerca* Diesing (= *C. brachyura* Lespés), *C. linearis* Lespés, *C. buccini* Lebour, and a new species *Cercaria Indica XXXVIII*. The morphology of this group was defined with the statement, "We unfortunately know nothing of the distribution and arrangement of the excretory tubules in any member of this group."

The cotylocercous cercariae are widely distributed, species occurring on both the Atlantic and Pacific shores of North America. Miller (1925 *a*) described *C. searlesiae* from Puget Sound, and (1925 *b*) at least three new species from Tortugas, Florida. One of the latter, *Cercaria* A of Miller, was shown by McCoy (1928, 1929) to be the larval stage of *Hamacreadium mutabile* Linton 1910. The cercariae develop in sporocysts in *Astraea americana*, encyst in the tissues of small fish (four different genera were employed successfully), and become sexually mature in the intestine and pyloric caeca of *Lutianus* (= *Neomaenis*) *griseus*. The next year McCoy (1930) showed that *Cercaria* B of Miller is the larva of *Hamacreadium gulella* Linton 1910. This cercaria encysts in small fish, *Thalassoma bifasciatum* and *Halichoeres bivittatus*, and develops to sexual maturity in fishes of the family Lutianidae. The relationship of the cotylocercous cercariae to trematodes of the family Allocreadiidae is thus established.

The lack of specific information and the possibility of errors of observation or interpretation in the older descriptions makes recognition of those species extremely difficult. The two species of Lespés and that of Pagenstecher would probably prove to be valid species if they could be recognised and redescribed. However, if one were to examine larval trematodes from the type hosts and type localities, as was done by Lebour, in an attempt to find and redescribe the species, it is doubtful if one could determine with certainty whether the cercariae found were the same as those formerly described.

Certain of the recent descriptions are also very unsatisfactory. Pelseneer (1906) gave no measurements and the specific name *C. pachycerca* Lespés, used by him, is impossible. The name must be either *C. brachyura* Lespés 1857 or *C. pachycerca* Diesing 1858, and since the species is not co-generic with *C. brachyura* Diesing 1850, the name of Lespés has priority. It is impossible at present to decide what species Pelseneer had before him. It is not impossible that he had more than one.

The redescriptions of *C. linearis* and *C. brachyura* as given by Lebour (1912) are confusing. Although she reported the cercariae from the same host species, her descriptions disagree so markedly from those of Lespés that one can hardly believe they were concerned with the same species. She found *C. brachyura* larger than *C. linearis*, a cleft stylet in *C. linearis* and none in *C. brachyura*, which is contrary to Lespés' original description. It is not at all certain that she had either of the species described by Lespés. Differences in host and in size of tail prevent the identification of *C. linearis* of Lebour with *C. brachyura* of Lespés. *C. brachyura* of Lebour is probably not identical with *C. brachyura* of Lespés. It may be identical with *C. cotylura* of Pagenstecher or with the species studied by Pelseneer. Lebour stated: "I think it is very likely to be a different species from Pelseneer's as his (*sic*) ventral sucker is larger than the oral and there are other small differences. It may be distinct from Lespés' species also, but they are all very much alike."

Sewell's description of *Cercaria Indica XXXVIII* is more complete and he made a beginning in the analysis of the excretory system, figuring five pairs of flame cells, two in the anterior and three in the posterior region of the body. This species is the only one of the cotylocercous cercariae known from a fresh-water host. The erection of a "Linearis" group has little to commend it however, and in the present state of our knowledge it appears wiser to retain the cotylocercous group, as characterised by Sewell.

The work of Miller and McCoy has solved an important problem in relating the cotylocercous cercariae to a definite group of adult trematodes. It is surprising however, that members of the family Allocreadiidae should have such different larvae as the cotylocercous cercariae and the several other types described by Looss (1894), Sewell (1922) and Brown (1927).

Two species of cotylocercous cercariae were found in *Gibbula umbilicalis* (= *Gibbus umbilicatus*), the most common snail on the algae of the flats at Roscoff. In view of the existing situation it is impossible to assign either of

them with certainty to any known species. They may be identical with the cercariae of Lespés and provisionally they are referred to those species although they do not entirely conform to Lespés descriptions and later knowledge may necessitate other disposition of them.

Cercaria linearis Lespés, **provis.**

(Figs. 3 and 3 *a*.)

This cercaria develops in orange-red sporocysts that fill the interlobular areas of the digestive gland and to a large extent replace that organ. Such sporocysts were described by both Pelseneer and Lebour. The sporocysts are active as noted; they vary from 1 to 2 mm. in length and from 0·4 to 0·6 mm. in width.

The cercariae (Fig. 3, p. 325) after emergence from the snail, creep along the bottom of the container by alternate attachment of the oral sucker and tip of the tail. At times the larvae attach themselves also by the acetabulum and frequently wriggle through débris on the bottom by a serpentine or eel-like movement. When undisturbed they attach themselves by the end of the stumpy tail; the bodies then stand upright in the water and wave back and forth. The appearance of the cercaria may readily suggest that "it is looking out for its intermediate host" as stated by Lebour, and although the habit may be of assistance in reaching a suitable intermediate host, I would hesitate to explain the behaviour on such a basis. The larvae do not encyst in the water and die after 24–36 hours.

The body as well as the tail of the cercaria is capable of much extension and retraction. Fully mature, normally emerged cercariae vary considerably in size. The body is 0·2–0·4 mm. long by 0·08–0·12 mm. wide. The specimen shown in Fig. 3 is somewhat compressed, and very large cercariae, flattened for study of the excretory system, may attain a length of 0·5 mm. and a width of 0·2 mm. The tail is about 0·05 mm. long and about 0·05 mm. in greatest breadth, although an increase in either proportion causes a corresponding decrease in the other. The central portion of the caudal end of the tail is protrusible and retractile, forming an adhesive organ. In addition, the tail is filled with gland cells. Cell outlines were not distinct, but the secretory granules stain a deep blood red with neutral-red. Presumably the secretion is sticky and aids in adhesion as the erect larva bends back and forth. The acetabulum is situated slightly behind the middle of the body and measures 0·07–0·09 mm. in diameter. The cuticula is not spined. The larva bears numerous, scattered, brownish patches, probably the secretion of dermal glands which lie below the body wall. There are also innumerable, minute, refractive spherical bodies distributed throughout the parenchyma. The region between the pharynx and the acetabulum contains fourteen large, lobed, penetration glands. They stain faintly with neutral-red and their nuclei may

then be counted. Ducts from these glands pass forward to open at the anterior tip of the body. The stylet (Fig. 3 *a*) is simple, 0·016–0·018 mm. long.

The oral sucker is the same size or slightly smaller than the acetabulum. It measures 0·07–0·075 mm. in diameter. There is a long prepharynx and the pharynx is 0·02–0·024 mm. long by 0·012–0·016 mm. wide. When the anterior end of the cercaria is retracted, the pharynx lies transversely across the body. Digestive caeca were not observed.

The excretory system was the special object of study as it has not been described previously for any member of the group. As reported for other species, the excretory vesicle is large and sac-like, occupying much of the region between the acetabulum and the caudal end of the body. It is lined with very large, apparently secretory cells, which project into the lumen. The vesicle opens posteriorly and I was unable to determine whether any portion of the excretory system extends into the caudal appendage. Collecting ducts, lined with cilia, pass forward from the vesicle and near the level of the cephalic margin of the acetabulum divide into anterior and posterior branches. Each branch is again subdivided to produce four tubules which end in flame cells. The location of the flame cells is shown in the figure although the tubules there shown were not all observed. There are however, eight flame cells on each side of the body and the flame cell pattern is $2\ (2 + 2) + (2 + 2)$.

The reproductive organs are not developed in the cercaria. A cluster of deeply staining nuclei, situated dorsal and anterior to the acetabulum undoubtedly represents the anlagen of the terminal portions of the genital ducts and copulatory organs.

The species here assigned to *C. linearis* Lespés differs from the original description of Lespés in only two important features. He found the species in the renal organ of *Littorina littorea*, but since he reported the cercariae in great numbers, other organs may have been infested. Presumably the parasite may develop in more than one snail host, and the difference in host does not preclude the assignment of the present species to *C. linearis*. The other difference pertains to the number of penetration glands. Lespés reported four cells, each with its own duct. It is possible that his account is suggestive rather than accurate, and one must bear in mind that the observations were made with the equipment of seventy-five years ago. Frequently in cercariae, ducts from two or more penetration glands follow the same course or may be associated in a common bundle. In the present species, the ducts from the penetration glands tend to follow four courses; one set of ducts passes on each lateral face of the oral sucker and on each side another group crosses the sucker between the lateral face and the midline. The gland cells are lobed and it is difficult to count them. When lobes overlie each other, cell boundaries are hard to follow, and I determined the number of cells by counting their nuclei when stained with neutral-red.

Accordingly there seems a strong probability that the species here described is actually *C. linearis* Lespés.

Cercaria brachyura Lespés, **provis.**

(Figs. 4 and 4 *a*.)

This cercaria was found together with the one just described in a dish that had contained one hundred specimens of *Gibbula umbilicalis*. The snails were left by the window. During the day the sun shone brightly and the water in the dish became warm. When the water was changed in the evening the snails were inactive, but it was thought they might revive in fresh, cool, sea water. Next day they were not only dead, but so decomposed that sporocyst stages of the trematode could not be obtained. Consequently the stages in the snail are unknown and the description is based entirely on the cercaria.

This species (Fig. 4, p. 325) is very similar to the one described as *C. linearis*, although the two types may be recognised by constant and important differences. In this species the brownish spots of the body wall are absent or only slightly developed, the larvae tend to creep rather than to stand erect, the acetabulum is slightly farther posteriad, and the form of the stylet (Fig. 4 *a*) is distinct and diagnostic.

These larvae do not differ appreciably in size from those referred to *C. linearis*. The body is 0·2–0·35 mm. long by 0·08–0·14 mm. wide. The specimen shown in the figure is slightly flattened. The tail varies in length from 0·035 to 0·07 mm. and the width is about equal to the length. The tail is very similar to that of the previous species and contains similarly staining gland cells.

The stylet (Fig. 4 *a*) is cleft anteriorly with four points directed anterolaterally, two on each side. The anterior points are larger than the posterior ones. The suckers are about the same size, 0·06–0·08 mm. in diameter. The specimens did not stain well with neutral-red and it was not possible to determine the number of penetration glands with certainty. They are lobed and their appearance is represented in the figure. The excretory and reproductive systems do not differ essentially from those of the previous species. The two cercariae are almost certainly members of the same genus and consequently form differences in organ systems are not to be expected.

The specimens provisionally referred to *C. brachyura* Lespés do not differ in important features from the original description. They are from a closely related if not from the same host species and the size limits overlap. The relative differences in length of body and tail are notable, but may be explained by differences in degrees of contraction. The cleft stylet of this species suggests a stylet with lateral projections as described by Lespés.

Since McCoy (1928, 1929, 1930) has shown that the cotylocercous cercariae are larvae of allocreadiid trematodes, it is interesting to compare the excretory system of these cercariae with the same system in other known or supposed allocreadiid larvae. The excretory formula of the cotylocercous cercariae described in this paper, 2 (2 + 2) + (2 + 2), is in agreement with the portion of the system reported by Sewell for *Cercaria Indica XXXVIII*, the only other

cotylocercous cercaria in which anything of the excretory system is known. Looss (1894) described the excretory system of *Allocreadium isoporum* and showed it to have the formula 2 (4 + 4 + 4) + (4 + 4 + 4). Faust (1924) gave the formula of *Acrolichanus petalosa* as 2 (2 + 2) + (2 + 2 + 2 + 2). Brown (1927) reported on the life history of *Crepidostomum farionis*. Metacercariae removed from the larvae of the May-fly, *Ephemera*, had an excretory pattern of 2 (2 + 2) + (2 + 2 + 2 + 2), the same as that reported by Faust for the closely related genus *Acrolichanus*. Brown believed, without experimental proof, that a cercaria described from a lamellibranch mollusc was the larva of *Crepidostomum farionis*. The cercaria had a flame cell formula of 2 (2 + 4) + (4 + 3 + 3 + 3) and developed in a redia with the excretory formula 2 (2 + 3) + (3 + 3 + 2). The difference between excretory systems in cercaria and metacercaria, and the lack of experimental evidence, suggests that the cercaria studied by Brown may not be the larva of *C. farionis*.

Many other cercariae have been suggested as larvae of the allocreadiid trematodes. Looss (1894) described a cercaria that he believed to be the larva of *Allocreadium isoporum*, although experimental proof was not obtained. The cercaria had eye spots and a large, powerful, tail. Sewell (1922) outlined an "Isopori" group of distome cercariae in which he included *C. isopori* of Looss and *Cercaria Indica XLIX*. The latter species has a large tail but no eye spots. It has an excretory pattern of 2 (2+2)+ (2+ 2), which is different from that of *A. isoporum* but agrees with that of the cotylocercous cercariae. Sewell noted further that *Cercaria Indicae XLIX* has a peculiar stylet which in form and position recalled that of the cotylocercous cercariae. Cort (1918) described *Cercariaeum mutabile* with an excretory formula of 2 (4 + 4 + 4 + 4) + (4 + 4 + 4 + 4), and on the basis of resemblance in the excretory and other systems suggested that it was the larval stage of a species of *Allocreadium*. Faust (1930) described *Cercariaeum sinense*, another tailless form with an excretory formula of 2 [2 (2 + 2) + 2 (2 + 2)], and referred it also to the family Allocreadiidae. It seems hardly possible that all of these different types can be larval stages within a single family.

NEW SPECIES OF CERCARIAE.

Cercaria roscovita n.sp.

(Fig. 5.)

This species develops in lemon coloured sporocysts that occupy the lymph spaces and interlobular areas of the digestive gland of *Littorina saxatilis nigrolineata*. When freed in sea water, the sporocysts are 0·7–1·5 mm. long by 0·25–0·45 mm. wide. Each sporocyst contains many, twenty to sixty, cercariae in various stages of development.

Fully developed, naturally emerged, cercariae (Fig. 5, p. 335) are elongate oval, and when extended the caudal end may appear truncated. The body is 0·15–0·3 mm. long by 0·06–0·12 mm. wide. The tail is small, subterminal in

attachment, and unless much extended it is shorter than the body. It may be 0·3 mm. long when slender and lash-like, and it may contract until it is only 0·08 mm. long. In swimming the body is contracted, bent ventrally, and lashing of the tail drives the larva upward and forward in the water. When the tail is motionless, the larva slowly sinks. The cercariae are not strong swimmers, and die without encysting on the bottom of the dish. The acetabulum, about the same size as the oral sucker, is situated slightly behind the middle of the body. The cuticula is armed with small spines and the oral sucker bears a small stylet. The stylet points directly forward, stains black with iron haematoxylin; it is 0·016–0·018 mm. long by 0·002–0·003 mm. wide.

The internal structures are difficult to observe in the mature cercariae, since the body contains so many gland cells. The numerous cystogenous cells stain with neutral-red in living specimens and with eosin in fixed and stained material. In the region just anterior to the acetabulum there are several gland cells whose ducts pass forward on either side to open at the anterior end of the body. They stain a diffuse faint pink with neutral-red and secretory granules are not distinct. These cells correspond to the stylet, salivary, or penetration glands of various authors. The parenchyma contains innumerable refractive droplets and near the posterior end there are several large gland cells whose ducts appear to open on either side near the caudal end of the body. They are brownish, do not stain with neutral-red unless strong solutions are used, and contain distinct secretory granules.

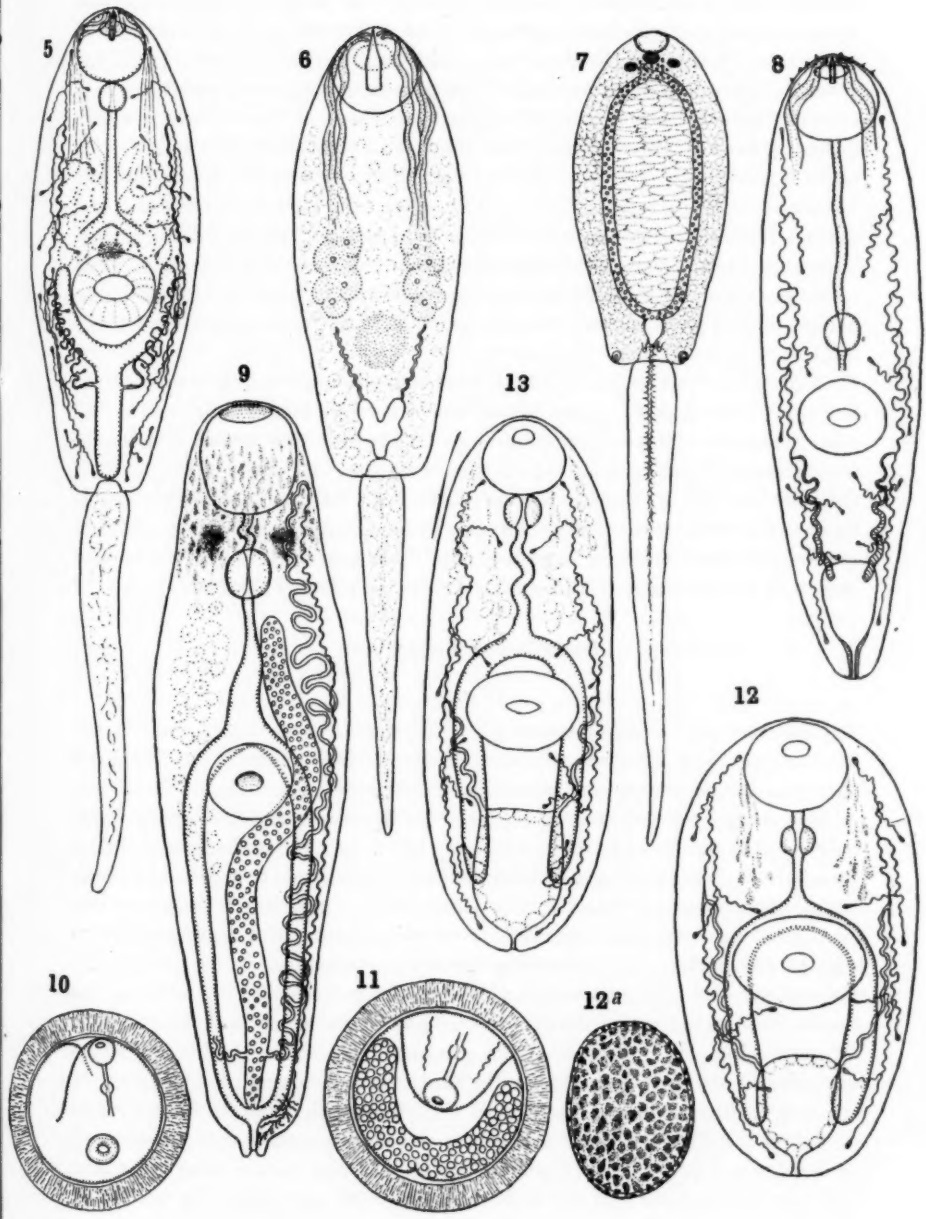
The oral sucker measures 0·042–0·05 mm. in living specimens and 0·034–0·045 mm. in fixed and stained ones. It is followed almost immediately by the pharynx, 0·014–0·018 mm. in diameter, and there is a long oesophagus, the alimentary tract bifurcating a short distance in front of the acetabulum.

The excretory system has been traced from early stages in the developing cercariae. The constriction which forms the tail of the larva occurs at the level of the excretory pores, and when it appears there are two flame cells, one anterior and the other posterior to the acetabulum, on each side of the body.

Capillaries from these cells meet at the acetabular level to form a larger tubule which passes in a coiled manner to the excretory pore. The terminal portions of these tubules are enlarged and by the time they join to form the beginning of the vesicle, there are two flame cells in the anterior and two in the

Explanation of Text-figs. 5–13.

Fig. 5. *Cercaria roscovita*; entire specimen, slightly compressed.
Fig. 6. *Cercaria ubiquitoides*; entire specimen, slightly compressed.
Fig. 7. *Cercaria lebouri*; entire specimen, slightly compressed.
Fig. 8. *Cercariaeum reticulatum*; entire specimen, not compressed.
Fig. 9. *Pharyngora bacillaris*; metacercaria, slightly compressed.
Fig. 10. *Spelotrema* sp.; cyst from *P. longicornis*.
Fig. 11. *Spelotrema* sp.; cyst from *C. maenas*.
Fig. 12. *Metacercaria galatheae*; specimen much compressed.
Fig. 12 *a*. *Metacercaria galatheae*; cyst from thoracic muscles of crab.
Fig. 13. *Metacercaria porcellanae*; specimen slightly compressed.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posterior half of each side of the body. Development continues until the definitive form of the system shown in the figure is attained. The system was worked out in young cercariae removed from sporocysts as there is no hope of tracing the details after the gland cells become filled with secretions. In mature cercariae when much flattened, the cystogenous material and stylet were extruded. The excretory vesicle is Y-shaped and opens at the caudal end of the body. It has an epithelial lining and the crura extend forward on either side of the acetabulum. From the median stem of the Y, near its bifurcation, collecting ducts pass laterad and forward on either side. They are much coiled and at the level of the acetabulum each divides into anterior and posterior branches. These branches in turn subdivide so that each drains three groups of flame cells. Each group contains three cells and the manner of branching is similar in anterior and posterior halves of the body. The flame cell formula accordingly is 2 (3 + 3 + 3) + (3 + 3 + 3).

This species belongs to the Xiphidiocercariae and agrees in general with the features of the Armata group. It differs from these larvae, however, in the communication of the collecting tubules with the excretory vesicle. Ordinarily the collecting ducts open into the tips or along the sides of the lateral crura of the vesicle, while in this species the ducts open into the median stem. The flame cell pattern corresponds with that of the family Plagiorchidae and presumably the larvae belong in that group. They are similar to *C. emasculans* and to *C. brevicauda* of Pelseneer, but cannot be referred to either species.

Cercaria ubiquitoides n.sp.

(Fig. 6.)

This cercaria, which resembles *C. ubiquita* Lebour, was found in the digestive gland of *Littorina rudis* and *L. littorea*. It develops in small, colourless, spherical to oval sporocysts which are 0·25–0·45 mm. long.

The normally emerged cercaria (Fig. 6, p. 335) is very small, the body being only 0·1–0·24 mm. long by 0·034–0·07 mm. wide. It is very delicate and almost transparent. The tail is small, subterminal in attachment, and unless much extended, it is shorter than the body. Extended, it measures 0·25 mm.; when contracted it is 0·06 mm. long. The larvae swim upward and when motionless, sink to the bottom. In swimming the body is contracted, bent ventrally, motionless, while the tail lashes violently. These cercariae die without encysting, on the bottom of the container. They cannot creep well since there is no acetabulum. It is possible that a group of cells, situated ventrally about one-third of the body-length from the posterior end, represents the anlage of the acetabulum. This region in side view frequently appears as a ventral protuberance. The cuticula of the body is armed with small spines and the oral sucker bears a large, sharply pointed stylet. The oral sucker measures about 0·03 mm. in diameter and the stylet is about 0·025 mm. long. No portion of the alimentary tract, other than the oral sucker, was observed.

The body contains many scattered cystogenous cells and on either side, near the middle, there are four lobed gland cells whose ducts pass forward to open at the anterior end of the body. The cells do not stain with neutral-red but the secretory granules are clearly visible. The ducts from three of the glands are associated in a common bundle that passes along the lateral face of the oral sucker, while the other duct crosses the sucker as shown in the figure. The secretory granules, colourless in the gland cells, stain a deep blood red in the terminal portion of the ducts and frequently accumulate there to form enlargements.

The details of the excretory system were not worked out. As the water evaporated, pressure of the cover glass flattened the larvae so that the vesicle and collecting ducts became visible for a moment, but the cercariae are so delicate that they do not withstand further pressure without rupture of the tissues, when of course the excretory system disappears. The vesicle is bifid, changeable in form. The excretory pore is situated at the caudal end of the body and collecting ducts pass forward from the crura of the vesicle almost to the level of the penetration glands.

This larva certainly belongs to the "Ubiquita" group of monostome cercariae as outlined by Sewell. Their separation from other Xiphidiocercariae is open to question, since, as has been shown in certain trematode groups (Stunkard, 1923), the presence or absence of an acetabulum is not a character of great systematic importance. Furthermore, the discovery of the life cycle of *Cryptocotyle lingua* (Stunkard, 1930 *a*) shows that a "monostome" cercaria later develops an acetabulum within a genital atrium and becomes a distome belonging to the family Heterophyidae.

Lebour (1912) believed *C. ubiquita* to be the larval stage of a species of *Spelotrema*, also a member of the family Heterophyidae. She traced the penetration of the larvae into the common green crab, *Carcinus maenas*, and their development into *Spelotrema*-like metacercariae. Concerning the cercaria she stated, "No ventral sucker is to be seen. It apparently develops afterward as does also the alimentary canal."

The ventral protuberance of *C. ubiquitoides* is apparently very similar to a corresponding structure in *Cercaria Indica LII* and *Cercaria Indica LXI*, which according to Sewell, "occupies the position of the acetabulum, but certainly is not a true sucker; it is connected with a mass of small rounded cells, which appears to be the rudiment of the genital organs, and possibly this papilla is the 'anlage' of the genital orifice." Since it is definitely established that the family Heterophyidae contains one "monostome" cercaria (*Cryptocotyle*), it appears very probable that Miss Lebour's suspicion is well founded, that the ventral protuberance of the "Ubiquita" monostomes develops into an acetabulum, and that these cercariae are larvae of *Spelotrema* and related genera.

Cercaria lebouri n.sp.

(Fig. 7.)

This species develops in rediae which occupy the interlobular lymph spaces of the digestive gland of *Littorina obtusa* and *L. littorea*.

The cercaria (Fig. 7, p. 335) is a good swimmer, but when on the bottom of the container it is sluggish and creeps by the use of the locomotor pockets at the postero-lateral corners of the body. These pockets are ventral in position and agree in structure with those described for other monostomes. When contracted, the body is almost as wide as long and in naturally emerged cercariae measures about 0·28 mm. long by 0·26 mm. wide. An extended specimen becomes long and slender with parallel sides and one measured 0·55 × 0·086 mm. Ordinarily the cercariae measure about 0·45 × 0·2 mm. The tail is attached subterminally and is slightly longer than the body. It measures 0·4–0·7 mm. long and 0·05–0·07 mm. in greatest width. The base of the tail performs nervous twitchy movements as the larva creeps. The longitudinal and circular muscles of the tail are conspicuous. There are three deeply pigmented eye spots. Their edges are irregular and measurements not precise, but the lateral ones are about 0·022 mm. while the anterior median one is about 0·033 mm. in diameter. Other scattered pigment granules are present near the eye spots. The cuticula is smooth, without spines.

The body is filled with gland cells which to a large extent obscure all other structures. The contents of these glands render the cercaria yellowish or brownish. They are of two different types; those outside of the ring formed by the excretory ducts stain intensely with neutral-red while those within the area remain yellowish.

The oral sucker measures about 0·05 mm. in diameter. The alimentary system is of the usual triclad type but development of the gland cells makes observation of the digestive organs exceedingly difficult. No pharynx was observed and the caudal ends of the digestive caeca were not distinct.

Only the terminal portions of the excretory system were traced. The vesicle is small, oval to triangular in shape, and the pore is posterior. There is apparently an excretory tubule which extends backward from the vesicle in the central region of the tail. From the vesicle, collecting ducts pass forward on either side of the body and unite near the anterior end to form a continuous ring. This structure is filled with refractive, concretionary bodies.

The cercaria belongs to the group of trioculate monostomes as characterised by Sewell. It is very similar to if not identical with the species from *Paludestrina stagnalis* described by Lebour (1912) as *C. ephemera* Nitzsch. As pointed out by Sewell, the identification of Lebour is not correct, since there are marked differences between the species she studied and *C. ephemera* Nitzsch. Furthermore, *C. ephemera* Nitzsch was described from *Planorbis corneus*, a fresh-water snail at Halle, Germany, whereas *C. ephemera* Lebour develops in a marine species.

Cercariaeum reticulatum n.sp.

(Fig. 8.)

This tailless larva (Fig. 8, p. 335) develops in the digestive gland of *Nassa reticulata*.

It is 0·28–0·57 mm. long by 0·075–0·14 mm. wide. The cuticula is armed with small spines and the cephalic end bears several minute papillae, each of which terminates in a bristle. The acetabulum is located slightly behind the middle of the body and measures 0·068–0·076 mm. in diameter.

The body contains cystogenous glands and the parenchyma is filled with refractive globules which interfere with the observation of internal structures. There are gland ducts which open at the sides of the stylet, but the number and exact position of the cell bodies could not be determined. The oral sucker measures 0·076–0·085 mm. in diameter and contains a small stylet. The stylet has the form of a cylinder which bears an elongated cone at its anterior end. The base of the cone is slightly narrower than the diameter of the cylinder. There is a long prepharynx; the pharynx measures 0·03–0·04 mm. in diameter, and is situated a short distance in front of the acetabulum. Intestinal caeca could not be observed.

The excretory system was worked out completely. The vesicle is pyriform, narrower posteriorly, and this neck region is surrounded by a strong sphincter. Collecting ducts arise near the antero-lateral margins of the vesicle and pass forward to the acetabular level. The posterior ends of these ducts are enlarged and contain strong circular fibres. The collecting ducts divide to form anterior and posterior branches; then in each branch subdivision occurs and is repeated, forming four groups of two cells on each side of the body.

This species is very similar to *Cercariaeum lintoni* Miller and Northup 1926, which occurs in *Nassa obsoleta* on the Atlantic coast of North America. I have found the same excretory pattern in both species and the observations of Africa (1930) are in complete agreement. The two species differ however, in other essential respects. Palombi (1930) has worked out the life history of a similar tailless larva at Naples and found that the adult is *Diphterostomum brusinae*, a member of the family Zoogonidae. Because of morphological agreement between the larva of *D. brusinae* and *C. lintoni*, Palombi concluded that they are closely related species. It is regrettable that the excretory pattern of *D. brusinae* is unknown, and that a comparison of excretory formulae of the two species is not yet possible.

METACERCARIAE.

Pharyngora bacillaris (Molin).

(Fig. 9.)

The larval stage of this species was reported first by Nicoll (1910) who found it free in tow-netting. Further data, including observations on development and distribution were given by Nicoll (1914). Lebour (1916) found the

unencysted metacercariae at Plymouth in various medusae and in the stomach of the ctenophore, *Pleurobranchia pileus*. She made certain additions to the description of Nicoll and showed the excretory collecting ducts opening into the long vesicle near its posterior end. According to Lebour, the collecting ducts divide near their communication with the excretory vesicle, one branch passing backward and the other forward.

The same larva (Fig. 9, p. 335) was found attached to the wall of the gastric cavity of *Pleurobranchia pileus* at Roscoff. There appears to be no doubt that it is the metacercarial stage of *Pharyngora bacillaris*. My observations of the excretory system are not in entire agreement with those of Lebour. The vesicle is long and narrow, extending to the region of the pharynx, and the collecting ducts open into it near the posterior end as stated by Lebour. But I was unable to confirm her observation on the bifurcation of the collecting ducts. These ducts, after their emergence from the vesicle, appear to pass forward without branching, extending in a convoluted course to the region of the oral sucker where they turn backward. The recurrent tubule gives off branches to the flame cell groups. The manner of branching in the system was not determined, but the number of flame cells is very large, probably a hundred or more on each side of the body. The fifteen cells shown at the caudal end in the right side of the figure were observed as represented, although the capillaries were not traced with certainty.

Furthermore, although the observation was not checked by the examination of serial sections, the digestive caeca appear to open into the excretory vesicle about midway between the origin of the collecting ducts and the excretory pore. If this observation is confirmed, another species belonging to another family is added to the list (Stunkard, 1931) of trematodes in which the intestinal caeca communicate with the excretory vesicle.

Although the details have not been worked out, it is apparent that in form of vesicle, in origin, location, and manner of branching of the collecting ducts, and in number of flame cells, the excretory system of this species is notably different from that of the stumpy-tailed cercariae, which, according to the work of McCoy, belong to the same family. Such differences appear subversive to the current belief that closely related species have the same excretory formula and that members of a given family have similar excretory systems. It is of course possible that the family Allocreadiidae, as now outlined, is a heterogeneous group and should be divided. One is apparently forced either to this conclusion or to doubt the infallibility of the excretory system as a criterion for determining natural relationships.

Spelotrema spp., **provis.**

(Figs. 10 and 11.)

Encysted metacercariae which apparently belong to the *Spelotrema* group were found in the crabs, *Porcellana longicornis* and *Carcinus maenas*. These cysts are colourless, spherical, thick-walled, elastic, and very tough. They

consist of a thick, striated, outer layer and a thin, membranous, inner layer. I was unable to rupture the cyst wall and remove the larva without injuring it. It is possible that all of these metacercariae belong to the same species and that they are identical with those reported by McIntosh (1865) and later writers. Observed differences may be due to different stages of development, or to development in different hosts or in different organs of the same host.

Cysts removed from the muscles of *P. longicornis* (Fig. 10, p. 335) measured 0·33–0·38 mm. in diameter. The worm is thin, the cuticula is spined, the oral sucker measures 0·07–0·08 mm. in diameter, and the acetabulum measures 0·08–0·1 mm. Similar cysts were found in the gills of *C. maenas*.

Other larger cysts were abundant in the liver and throughout the body of *C. maenas*. These cysts (Fig. 11, p. 335) measure 0·4–0·5 mm. in diameter and the wall may be 0·075 mm. thick. The oral sucker in different specimens varied from 0·068 to 0·095 mm. in diameter and the acetabulum was about the same size. The cuticula is spined, and the excretory vesicle of the larger specimens contained concretionary bodies. The prepharynx, pharynx and beginning of the oesophagus do not differ except for size from those structures in metacercariae found in *P. longicornis*.

Metacercaria galatheae n.sp.

(Figs. 12 and 12 *a*.)

Heavily pigmented, oval cysts (Fig. 12 *a*, p. 335) were abundant in the crab, *Galathea squamifera* Leach, attached to the muscles and free in the body cavity. The pigment is deposited in the thick, rigid, outer wall of the cyst which is probably formed by the tissues of the host. Within, there is a thin membranous wall, formed from material elaborated within cystogenous glands of the larva. The cysts are 0·22–0·24 mm. long by 0·16–0·18 mm. wide. The outer wall may be easily broken and removed. The larva may then be freed on rupturing the membranous cyst under a cover glass by the application of gentle pressure.

The worm (Fig. 12, p. 335) is 0·2–0·3 mm. long by 0·14–0·18 mm. wide. The acetabulum is 0·07–0·1 mm. in diameter. The specimen shown in the figure was much flattened in the study of the excretory system. Remnants of former penetration glands are present in the preacetabular area. The oral sucker is 0·05–0·08 mm. in diameter. There is a short prepharynx, pharynx, oesophagus, and the alimentary canal bifurcates in front of the acetabulum, the caeca extending well toward the caudal end of the body. The excretory system was readily observed. The pore is caudal, the vesicle sac-shaped, and collecting ducts pass forward on either side to the level of the anterior margin of the acetabulum. Here each divides into anterior and posterior branches and the flame cell formula is $2\,(2+2)+(2+2)$, as shown in the figure.

The excretory pattern is like that of the stumpy-tailed cercariae from *Gibbula umbilicalis* and other structures are very similar. The morphological

agreement suggests close relationship, but only experimental evidence can show whether this is true or not.

Metacercaria porcellanae n.sp.

(Fig. 13.)

This species is very similar to the preceding one. The cysts were found in the crabs, *Porcellana platycheles* and *P. longicornis*. They are 0·26–0·32 mm. long by 0·18–0·2 mm. wide. The outer wall of the cyst consists of solid, homogeneous, yellow material, which breaks in pieces when the cyst is pressed. The inner wall of the cyst may then be ruptured, freeing the larva.

Such excysted metacercariae (Fig. 13, p. 335) are 0·2 mm. long when contracted and 0·6 mm. long when extended and flattened. They are ordinarily 0·18–0·2 mm. wide. The acetabulum is 0·1–0·12 mm. in diameter. Vestiges of gland cells are present. The oral sucker is 0·08–0·095 mm. in diameter. There is a short prepharynx, the pharynx is about 0·04 mm. in diameter, the oesophagus is long, the alimentary tract bifurcates just in front of the acetabulum, and the caeca extend to the middle of the excretory vesicle. The excretory pattern, as shown in the figure, is the same as that of the metacercaria from *Galathea squamifera*, and these larvae almost certainly belong to closely related species.

SUMMARY.

Seven species of cercariae and four of metacercariae collected at Roscoff, Finistère, during August, 1931, are described. They have been referred to appropriate systematic groups and wherever possible, probabilities concerning life histories are suggested. Four of the cercariae and two of the metacercariae are recorded as new to science.

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A STUDY OF SOME CERCARIAE OBTAINED FROM MOLLUSCS IN THE SUDAN.

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(With Plate XVI, containing 5 Figures.)

In a previous paper[1] we gave an account of three non-furcocercous cercariae found in *Bulinus contortus* in the Sudan. During further investigations on the intermediate hosts of Bilharzia, other cercariae have been found in molluscs collected in different districts of the Sudan. As their post-larval development has not been studied, the classification of these cercariae has not been attempted; the procedure of giving them numbers rather than names, indicating specific peculiarities, has been continued.

Cercaria sudanensis No. 4.
(Fig. 1.)

Host: *Physopsis didieri.*

Locality and date: Yei river. January, 1931.

This cercaria, when viewed *in vitro*, adopts alternate swimming and crawling movements. When swimming, the anterior portion of the body is curled ventrally and the tail displays a vigorous lashing movement; on coming to rest, the acetabulum adheres to the side of the glass tube and a crawling movement is brought about by alternate expansion and contraction of the body, the tail being drawn up to the position previously occupied by the body.

The comparatively short tail, with its ventral attachment, is composed mainly of longitudinal muscle fibres and a few parenchymatous cells enclosed within a thick cuticular membrane. The latter is completely surrounded by a fin fold. Between the fin fold and the cuticular membrane there are supporting rays which are more numerous towards the posterior extremity.

The tail is devoid of an excretal canal.

The body is 392–420 μ long by 172–180 μ broad. The tail averaged 290 μ in length and at its base 58 μ in breadth.

[1] *Parasitology*, **23**, 271–81, with 6 text-figs. (1931).

Surrounding the body is a well-developed spinose cuticle which is thickened at the site of attachment of the tail to represent lateral suctorial pockets. There are no flame cells either in the body or tail.

This cercaria develops in salmon pink coloured sporocysts in the liver.

Cercaria sudanensis No. 5.
(Fig. 2.)

Molluscan host: *Bulinus truncatus.*

Locality and date: Gezira irrigation canals. February, 1931.

This is the commonest of the furcocercous cercariae infesting *B. truncatus* in the Gezira.

When examined in water *in vitro*, it is found in all layers of the fluid, a characteristic feature being the white glistening appearance of the body. It lies suspended at any angle but most frequently in a vertical position, tail uppermost with furci curved slightly forwards. It swims by sharp but short dashes in any direction, usually tail foremost, cessation of movement being accompanied by extension of the furci with the anterior portion of the body curved ventralwards, producing a characteristic appearance similar to a hook. It frequently remained in a state of suspended activity for long periods.

The body is 160–220 μ long and averages 34 μ in breadth. The tail stem is 208–240 μ long and averages 31 μ in breadth.

The tail stem is very muscular with numerous parenchymatous cells that extend into each furcus.

Accurate length measurements for the furci were not possible owing to their curvature. Each approximated the length of the body. In breadth they measured at their base 18 μ, their extremities terminating in sharp points.

The anterior organ was pear-shaped and was limited posteriorly by a muscular collar through which passed two pairs of penetration gland ducts; these traversed the entire length of the anterior organ and terminated at the anterior extremity in hollow conical spines.

The anterior organ measured 52 μ in length and 32 μ in breadth, and was covered with rows of retrorse spines.

The mouth cavity was represented by a simple tube opening terminally that led posteriorly to a small bulbous muscular pharynx and rather wide oesophagus, the latter bifurcating into two thinner tubes which encircled the acetabulum and ended in saccular dilatations near the posterior end of the body.

There were two pairs of penetration glands each with a well-marked nucleus and granular cytoplasm; the posterior pair were more laterally situated and appeared to be more granular than the superimposed anterior pair that occupied the middle line. Extending forwards from the two pairs of penetration glands were a corresponding number of gland ducts that ended terminally in hollow spines.

The nervous system was represented by a collection of cells situated laterally about the level of the oesophageal bifurcation.

The muscular acetabulum, covered with spines, was situated in the posterior third of the body. It was capable of considerable protrusion, seen best in profile.

Posterior to the acetabulum there was a collection of cells representing the genitalia.

The excretory system was simple in type, consisting of an excretory vesicle with two lateral horns situated at the posterior extremity of the body. Connected with each horn is a convoluted tubule that could only be traced as far forwards as the anterior margin of the acetabulum. Neither flame cells nor ciliated areas were observed. There is a caudal canal communicating posteriorly with the excretory vesicle; it extends the whole length of the tail stem, and at the furcal junction divides into two branches each of which passes along the centre of the corresponding furcus to end in a sub-terminal excretal pore.

Cercaria sudanensis No. 6.

(Fig. 3.)

Host: *Physopsis globosa* (Morelet).

Locality and date: Rashad. February, 1931.

Observed in water *in vitro* this short non-furcocercous cercaria shows a preference for the upper layers of the fluid. It is a very active swimmer, rarely displaying periods of quiescence. When swimming, the body is curved towards the ventral aspect, and is contracted so as to form a glistening white spherical mass, which is driven through the water by vigorous lashing movements of the tail. When cessation of movement occurs, the cercaria sinks slowly in the medium. Mounted in water under a coverslip, it displays a crawling movement, the body exhibiting considerable powers of extension and contraction. The whole surface of the body and tail is covered with fine retrorse spines. The tail is provided with marked longitudinal and circular muscle fibres and parenchymatous cells; it has a ventral attachment and is completely surrounded by a caudal fin. The body in heat-killed specimens is oval, 168–200 μ long and 100–118 μ broad. Projecting from the anterior extremity is a spear-shaped stylet, which appears to arise from a sub-terminal mouth in the anterior organ. At times this stylet becomes tilted to one or other side. The sharp point of the stylet is supported on a shoulder. The stylet measures 28 μ in length. The muscular anterior organ measures 48 μ in diameter and possesses two cephalic glands. Posterior to the anterior organ there is a long prepharynx leading to a muscular pharynx; the oesophagus bifurcates at the anterior margin of the acetabulum into two caeca that terminate posteriorly to the acetabulum. These caeca are located with difficulty. The acetabulum is situated about the middle of the body, and is smaller than the anterior organ. The penetration glands, two on each side, are situated at the posterior end of the body; they possess a granular cytoplasm and a well-marked nucleus; their

sacculated ducts pass forwards to the sides of the anterior organ, and open near the stylet.

The excretory system is simple in type and is devoid of flame cells. It consists of a posterior sub-spherical sac connected by an isthmus to a wide sausage-shaped anterior sac. From each end of it there arises a thickened main excretory tube, which follows a convoluted course forwards to the level of the posterior margin of the anterior organ, curves outwards and backwards, and continues as a thinner tube that extends along the body margin passing behind the penetration glands to enter the posterior sac of the bladder. There are no eye spots. This cercaria develops from sausage-shaped sporocysts containing a brownish yellow pigment.

Cercaria sudanensis No. 7.
(Fig. 4.)

Host: *Bulinus truncatus*.

Locality and date: White Nile, Dueim. March, 1931.

When observed in water *in vitro* this furcocercous cercaria is found lying in parallel columns tail uppermost in all layers of the fluid. Its movements are short but rapid, progressing tail foremost either in an oblique or vertical direction or going round in circles. Cessation of movement is first accompanied by extension of the furci to right angles, and as the cercaria floats body downwards the furci slope posteriorly while the anterior portion of the body is carried ventralwards like a hook. When viewed with a lens, the body presents a white appearance. The whole cercaria is covered with retrorse spines, which appear to be more numerous on the body than on the tail stem. Mounted in water under a coverslip, the body shows great powers of extension and contraction, its anterior portion frequently bending to one or other side. The body is elongate-oval, 200–220 μ long and 72 μ broad; the tail stem measured 260 $\times$ 32 μ and the furcus 192–200 $\times$ 16 μ. The anterior organ is pyriform-shaped, and is limited posteriorly by a muscular collar through which pass the ducts of the penetration glands. The anterior third of the organ is covered with thick retrorse spines. The terminal mouth is connected posteriorly with a median tube that leads into a bulb opening into a muscular pharynx; connected with the latter is a short wide oesophagus, which bifurcates into two narrow tubes that extend posteriorly and terminate in two sac-like dilations near the excretory vesicle. There are two pairs of convoluted penetration glands each with a well-marked nucleus, the posterior pair being situated on a level with the acetabulum, the anterior pair lying anterior to the acetabulum. These glands are very granular and extend forward as convoluted sacs as far as the bifurcation of the oesophagus where they merge into two pairs of gland ducts that pass through the anterior organ and terminate close to the mouth. The muscular acetabulum, capable of considerable protrusion, measures 32 μ in diameter, and is situated about the middle of the posterior half of the body;

its orifice is guarded by spines. The Y-shaped excretory vesicle is situated at the posterior extremity of the body; two main thick collecting tubules pass forwards from it, and at a point midway between the pharynx and the acetabulum each divides into anterior and posterior collecting tubules. Each anterior collecting tubule extends forwards, and gives off three flame cells, and each posterior collecting tubule extends backwards, and gives off a similar number of flame cells. In addition there are three ciliated areas on each posterior collecting tubule. It is difficult to trace the ultimate termination of each posterior collecting tubule, but it appeared to connect with the excretory vesicle. The caudal excretory canal opens into a bladder. It traverses the whole length of the tail stem, and at the furcal junction divides into two branches each of which passes along the centre of the corresponding furcus to end in a terminal excretal pore. There are four flame cells in the tail stem.

The tail stem has a ventral attachment, and possesses well-marked longitudinal muscle fibres and parenchymatous cells; the latter extend into the furci. Unstained preparations show clear hyaline masses on the sides of the tail stem; these appear to represent caudal glands.

This cercaria develops in grey-coloured tubular sporocysts measuring 5 mm. in length.

Cercaria sudanensis No. 8.

(Fig. 5.)

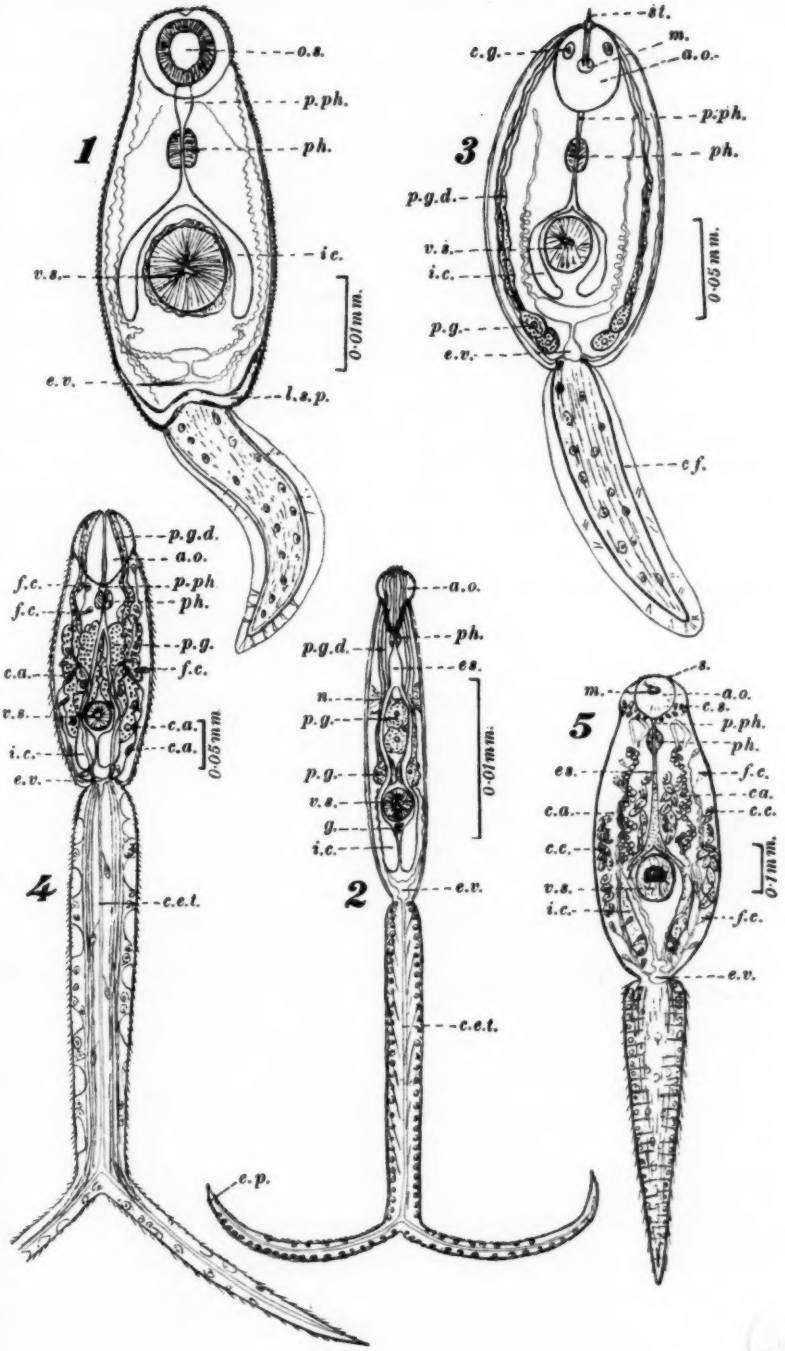
Host: *Bulinus truncatus.*

Locality and date: Fasher. March, 1931.

Observed in water *in vitro* this is an actively swimming pigmented cercaria found in all layers of the fluid; in swimming, the ventrally curled body is driven through the water by means of vigorous lashing movements of the tail, the cercaria going down to the bottom of the fluid, and rising rapidly; at times it adopts rapid crawling movements. It rarely shows quiescent periods.

The body has considerable powers of extension; in the contracted state, it is more or less oval, 0·5 mm. long and 164–180 μ broad at its widest part. Beneath a fairly thick cuticle are large numbers of oval cystogenous cells containing greyish brown granules, which account for the pigmented appearance of the body. The rounded anterior extremity is occupied by the muscular oral sucker, which measures 60 μ in diameter. The mouth is sub-terminal, and is guarded by spines. There is a definite shoulder, and ranged along it a layer of spines pointing backwards. Posterior to the oral sucker there is a short prepharynx leading into a muscular pharynx. Behind the pharynx, there is a long oesophagus, whose lumen widens as it approaches the anterior margin of the acetabulum where it bifurcates into two intestinal caeca that terminate close to the excretory vesicle. The very muscular acetabulum 60 μ in diameter is situated behind the mid-point of the body; its funnel-shaped opening is protected by spines.

The excretory vesicle consists of a posterior sac connected by an isthmus to a smaller anterior sac. Extending forwards from the latter are a pair of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convoluted tubules, that can be traced to the level of the anterior margin of the acetabulum, where they appear to connect directly with a series of round refractile excretory granules that extend to the level of the pharynx. At this point the convoluted tubules become evident again, each running forwards; for a short distance their subsequent course cannot be followed, eventually they reappear as fine ducts extending backwards to terminate in the posterior sac of the excretory vesicle. Each duct gives off capillaries supporting eight flame cells; two of these flame cells are apparent in the anterior third of the body, and the remainder occur in the posterior third close to the excretory vesicle. In the middle third each duct shows six ciliated areas.

There is no caudal excretal canal. The cone-shaped tail is capable of considerable extension, and has a ventral attachment. It measures 0·5 mm. in length and at its base 56–60 μ in breadth. Beneath the spine-covered cuticle there are well-developed transverse and longitudinal muscle fibres and parenchymatous cells; the extreme tip of the tail is devoid of cells.

This cercaria develops in sausage-shaped, orange-coloured rediae measuring 1 mm. × 192 μ in breadth. The redial sac is filled with yellow pigment granules and cercariae, and contains a birth pore and lateral locomotor appendage. The mouth is terminal, and leads back to a well-marked pharynx opening into a rhabdocele gut.

This cercaria is capable of encystment. The cysts are circular with a double contour, and measure 152 μ in diameter.

EXPLANATION OF PLATE XVI.

The figures represent camera lucida drawings of specimens in serum.

Fig. 1. *Cercaria sudanensis* No. 4.
Fig. 2. *Cercaria sudanensis* No. 5.
Fig. 3. *Cercaria sudanensis* No. 6.
Fig. 4. *Cercaria sudanensis* No. 7.
Fig. 5. *Cercaria sudanensis* No. 8.

Lettering:

a.o. anterior organ; *c.a.* ciliated areas; *c.c.* cystogenous cell; *c.e.t.* caudal excretory tube; *cf.* caudal fin fold; *c.g.* cephalic gland; *c.s.* collar spines; *e.p.* excretory pore; *es.* oesophagus; *e.v.* excretory vesicle; *f.c.* flame cell; *g.* genitalia; *i.c.* intestinal caecum; *l.s.p.* lateral suctorial pocket; *m.* mouth; *o.s.* oral sucker; *p.g.* penetration gland; *p.g.d.* penetration gland duct; *ph.* pharynx; *p.ph.* prepharynx; *st.* stylet.

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TRICHODECTIDAE (MALLOPHAGA) FOUND ON AFRICAN CARNIVORA

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(With 9 Figures in the Text.)

Most of the new species herein described were collected by me from skins in the Transvaal Museum, Pretoria, for the loan of which I am deeply indebted to Mr Austin Roberts.

In most species of Mallophaga, as Harrison (1915) pointed out, there are six pairs of abdominal spiracles, situated either on segments 3–8 or 2–7. In a number of species of Trichodectidae parasitic on carnivora, however, there are only three pairs of abdominal spiracles present, and in at least three species, including *Trichodectes nasuatis* Osborn, found on *Nasua narica*, an American carnivore, they are absent. Harrison (1915) stated that he could only detect five pairs in *Trichodectes divaricatus* Neumann, and according to Stobbe (1913) there are only four pairs in *Felicola viverriculae*. The only other exceptions known are to be found in the species belonging to the sub-family Gliricolinae and the family Trimenoponidae. In both these groups, the species of which are also parasitic on mammals, there are only five pairs of abdominal spiracles, these being present on segments 3–7.

In addition to the abdominal spiracles the Trichodectidae also possess a pair of prothoracic spiracles. The only other species known to possess these belong to the family Gyropidae and also to the genus *Tetrophthalmus*, which includes a few species parasitic on pelicans.

Harrison (1915) states that it is reasonable to conclude that the difference in stigmatal arrangement is due to the suppression of the first abdominal segment or its incorporation with the thorax, and that this condition is strongly suggested in *Trichodectes*. He found that the segments in front of those which bear the stigmata usually show small scars in the position which the spiracles would have had, the scars indicating that the Mallophaga, or their ancestors, originally had two additional pairs of abdominal stigmata. His argument does not, however, explain either the entire absence of abdominal spiracles or even the presence of only three or four pairs. He states that he examined about twenty species of Trichodectidae, but it is very evident that these did not include either *Felicola subrostrata* (Nitzsch) or any of the species parasitic on mongooses.

Harrison, in the same paper, compared the diameter of abdominal spiracles of species belonging to different genera, and found that the stigmatic apertures

of the Ischnocera were considerably smaller than those of the Amblycera. However, in the Trichodectidae, which is included in the Ischnocera, the abdominal spiracles frequently vary in size, even in closely allied species of about the same size. Furthermore, in at least one species, namely *Felicola acutirostris*, the first pair of spiracles is larger than the two remaining pairs. Also, those of the females are usually larger than those of the males, but as the females are larger than the males, this is only to be expected.

Harrison (1915) stated: "The general constancy of the respiratory system in Mallophaga seems to be a good argument for allowing a fair amount of importance to such differences as occur." He considered that the number of abdominal spiracles present is at least of generic, if not of family importance. We have seen that the number of abdominal spiracles present varies in the Trichodectidae parasitic on Carnivora, and the question arises, can they be considered of generic importance in this family?

It will first of all be necessary to explain that the parasites of carnivora can be divided into two very distinct groups, namely, one group parasitic on Canidae, Mustelidae and the American Procyonidae, and the other on Felidae and Viverridae. There are, however, two species found on Viverridae, viz. *Trichodectes madagascarensis* Mjöberg and *Suricatoecus cooleyi* (Bedf.) which belong to the first group. *Protelicola intermedia* n.gen. et sp., the parasite of the aardwolf, *Proteles cristatus*, is intermediate between these two groups, and it is interesting to note that this host is the only animal included in the family Protelidae, which mammalogists place between the Canidae, Mustelidae, etc., on the one hand, and the Felidae and Viverridae on the other.

In both these groups the number of abdominal spiracles present is variable. As no mention is made of these in most descriptions, and they are shown only in one or two figures, I can, for the most part, only refer to those of species I have examined. In the first group there are six pairs present in all the species recorded from African hosts, excepting *Suricatoecus cooleyi* (Bedf.), which possesses only three pairs. In *Trichodectes octomaculatus* Paine, parasitic on Procyonidae, there are, according to Neumann's figure, only three pairs present. In *Trichodectes nasuatis*, also parasitic on Procyonidae, they are absent.

In the second group, most species of *Felicola* known to be parasitic on cats and mongooses possess three pairs of abdominal spiracles. *F. caffra* (Bedf.), a typical cat parasite, however, possesses six pairs of abdominal spiracles, and has a longitudinal sclerite on each side of the male genitalia. These sclerites have probably no generic importance as similar sclerites may be present or absent in closely related *Trichodectes* spp. In both *F. genetta* (Bedf.) and *F. helogale* n.sp. the abdominal spiracles appear to be absent. The latter species also differs from other *Felicola* spp. in the shape of the head and gonopophyses of the female, but they are hardly sufficiently distinct to be considered of generic significance.

As *F. caffra* is far more closely related to *Felicola* spp. than it is to other

species possessing six pairs of abdominal spiracles, and as neither *F. genetta* nor *F. helogale* is closely related to *T. nasuatis*, the only other species known in which the abdominal spiracles are absent, I can only conclude that the structure of the respiratory system in the Trichodectidae is not, in the absence of other important characters, of generic significance.

It is of interest that the parasites of mongooses, excepting *S. cooleyi*, are more closely related to the parasite of the domestic cat than to those of genets, which mammalogists include along with the mongooses, in the family Viverridae. It is difficult to understand why *S. cooleyi*, the parasite of the suricate, should be so very distinct. Genets also harbour two very distinct types of parasites.

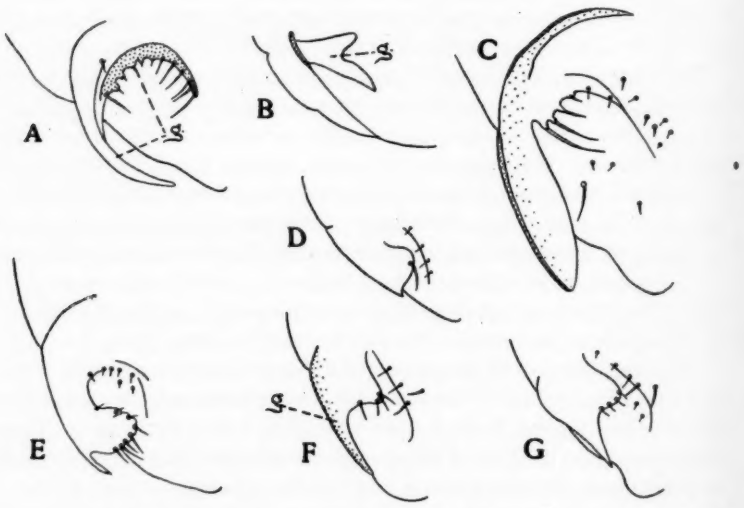

Fig. 1. Gonopophyses of females of: *A*, *Trichodectes ovalis* Bedford; *B*, *Suricatoecus cooleyi* (Bedford); *C*, *Protelicola intermedia* n.sp.; *D*, *Felicola helogale* n.sp.; *E*, *Felicola caffra* (Bedford); *F*, *Felicola setosa* n.sp.; *G*, *Felicola subrostrata* (Nitzsch).

The most important generic characters in the Trichodectidae appear to be:

(i) The shape of the head and its sclerites.

(ii) The presence or absence of sclerites on the tergites and sternites.

(iii) The gonopophyses of the females.

In several of the other families of Mallophaga the male genitalia are of generic significance, but in the family Trichodectidae they frequently vary considerably, even in closely allied species.

The antennae are three-segmented in both sexes in all the known species parasitic on Carnivora.

KEY TO THE GENERA.

1. Forehead short and broad, with anterior margin rounded, but a median notch may be present. 2.
   Forehead triangular or sub-triangular. 3.

2. Gonopophyses of female as in Fig. 1 *A*; abdominal spiracles present on segments 2–7 in African species. **Trichodectes** Nitzsch.
   Gonopophyses of female as in Fig. 1 *D*; abdominal spiracles present on segments 2–4. **Suricatoecus** n.gen.

3. Tergal plates absent in both sexes; gonopophyses of female as in Fig. 1 *C*. **Protelicola** n.gen.
   Tergal plates well developed in both sexes; gonopophyses of female with a well-developed lobe on inner margins. **Felicola** Ewing.

Genus **TRICHODECTES** Nitzsch.

1818. *Trichodectes* Nitzsch, p. 294.

Until recently most species belonging to the family Trichodectidae were placed in this genus. It should probably only include species parasitic on carnivores belonging to the Canidae, Procyonidae, and possibly Mustelidae.

Ewing (1929) erected the genus *Neotrichodectes* for *Trichodectes mephitidis* Packard, parasitic on an American skunk, but it is impossible to state at present whether it should be retained, and if so, what other species the genus should include.

Genotype: *Ricinus canis* De Geer (1778).

1. **Trichodectes canis** (De Geer).

1778. *Ricinus canis* De Geer, p. 81, pl. 4, fig. 16.
1838. *Trichodectes latus* Nitzsch, in Burmeister, p. 436.
1852. *Trichodectes octopunctatus* Leach, in Denny, p. 29.
1880. *Trichodectes latus* (Nitzsch) Piaget, p. 384, pl. 31, fig. 6.

This species has been recorded taken off domestic dogs in Europe, America and Australia, but so far has not been reported from these animals in Africa. Mr F. V. Collins, of Port Adelaide, informs me that he has found it on the Australian dingo.

2. **Trichodectes matschiei** Stobbe.

1913. *Trichodectes matschiei* Stobbe, p. 372.

Described from two females taken off *Lutra matschiei* in the Cameroons. It is closely related to *T. exilis* Nitzsch, a parasite of the European otter, *Lutra vulgaris*.

3. **Trichodectes ovalis** Bedford.

(Fig. 1 *A*.)

1928. *Trichodectes ovalis* Bedford, p. 841, pl. 1, figs. 1, 3; pl. 6, figs. 1, 3.

Described from females and males taken off *Poecilogale albinucha* at Onderstepoort. Bedford (1929) also recorded it taken off *Ictonyx striatus* in South-West Africa and Natal.

4. **Trichodectes vosseleri** Stobbe.

1913. *Trichodectes vosseleri* Stobbe, p. 371, fig. 2.

Described from male and female taken off *Potamochoerus demunis*, Tanganyika Territory. I have taken a male and female off *Mellivora capensis* (Schr.), Knysna, C.P. (Transvaal Mus. No. 1362), and have also received specimens from Mr J. Hewitt taken off the same host at Kleinpoort, Albany District, C.P. As this large species is a typical parasite of Mustelidae there can be no doubt that either an error was made in recording the host of the types, or the specimens were stragglers. I here designate the male as the holotype, no previous designation having been given.

5. **Trichodectes zorillae** Stobbe.

1913. *Trichodectes zorillae* Stobbe, p. 374, fig. 3.

Described from females and males taken off *Zorilla lybica* and *Z. vaillanti* in Tunis. I here designate the female as the holotype, no previous designation having been given.

6. **Trichodectes madagascariensis** Mjöberg.

1910. *Trichodectes madagascariensis* Mjöberg, p. 64, figs. 38, 39.

Described from a male taken off a civet cat, *Eupleres goudoti* in Madagascar. It is a very distinct species and will probably have to be placed in a new genus.

Genus **SURICATOECUS** n.gen.

Head wider than long, the anterior margin rounded, without a median notch. The lateral sclerites on forehead turn inwards for a short distance in front of the trabecula-like processes and project backwards in front for a short distance on each side of the meson. Temporal bands absent. Abdomen without tergal sclerites, except for a narrow transverse plate on the eighth segment in the female, and in the male there is a narrow transverse sclerite at the latero-anterior angles of the first tergite. Gonopophyses of female very characteristic, as shown in Fig. 1 *D*. Abdominal spiracles present on segments 2–4. Only the following species is included in this genus.

1. **Suricatoecus cooleyi** (Bedford).
(Fig. 1 *B*.)

1929. *Trichodectes cooleyi* Bedford, p. 513, fig. 13.

Described from males and females taken off the mongoose, *Suricata suricatta hamiltoni* in the Pretoria District, Transvaal.

Genus **PROTELICOLA** n.gen.

Head wider than long; forehead triangular, slightly notched in front; the lateral sclerites on each side turn inwards for a short distance in front of the trabecula-like processes. Temporal bands present, but not well developed.

Abdomen without tergal sclerites, except for a transverse plate on the eighth segment in the female. Gonopophyses of female characteristic, as shown in Fig. 1 *C*. Abdominal spiracles present on segments 2–7.

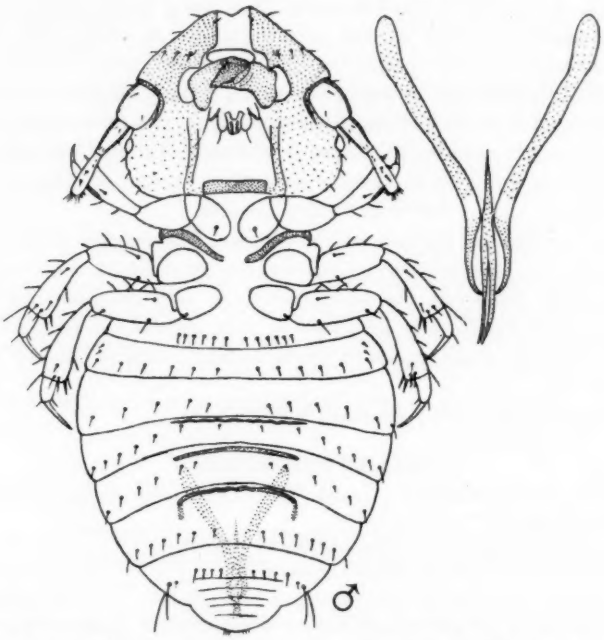

Fig. 2. *Protelicola intermedia* n.sp., ventral aspect and genitalia of ♂.

Only the following species is included in this genus.

1. **Protelicola intermedia** n.sp.
(Figs. 1 *C*, 2.)

Males and females taken off aardwolf, *Proteles cristatus* (Sparrm.), Umkomaas Valley, Natal, 19. vi. 1931 (coll. L. Hill). Holotype a male.

The head of this species resembles those of the majority of species parasitic on Felidae and Viverridae and the abdomen is similar to those of species found on Canidae and Mustelidae.

Male. Total length 1·22 mm. Head 0·36 × 0·42 mm. Forehead slightly asymmetrical in front. On the dorsum there is a transverse row of nine setae on the forehead, two on each side between the antennae and a median transverse row of four behind. Sternites 4–6 each with a median, narrow transverse sclerite. Genitalia as in Fig. 3.

Female. Total length 1·82 mm. Head 0·47 × 0·56 mm. Differs from the male in having a broadish transverse sclerite at the base of the eighth tergite, and a narrow, transverse sclerite is only present on the sixth sternite.

Genus **FELICOLA** Ewing.

1929. *Felicola* Ewing, pp. 122, 192.
1929. *Felicinia* Bedford, p. 519.

Forehead triangular or sub-triangular. Transverse tergal sclerites of female usually terminate in long, narrow, curved points at either end. They are slightly narrower in some species than in others, but it is doubtful whether these small differences are of systematic value. Gonopophyses of female with a well-developed lobe on the inner margins. Male genitalia frequently vary considerably, even in closely allied species. Abdominal spiracles usually present on segments 2–4 but may be entirely absent, or present on segments 2–5 or segments 2–7.

Species parasitic on Felidae and Viverridae. The females of the different species are for the most part very similar and often difficult, if not impossible, to identify. The males, on the other hand, are very dissimilar and easy to determine. Fortunately they are about as numerous as the females, and not extremely rare as in some species of Trichodectidae, such as *Bovicola bovis* (L.) and *B. equi* (L.).

Genotype: *Trichodectes subrostrata* Nitzsch.

KEY TO THE SPECIES.

1. Abdominal spiracles present. **2.**
   Abdominal spiracles absent. **10.**
2. More than three pairs of abdominal spiracles present. **3.**
   Three pairs of abdominal spiracles present. **4.**
3. Six pairs of abdominal spiracles present on segments 2–7. **F. caffra** (Bedf.).
   Four pairs of abdominal spiracles present on segments 2–5. **F. viverriculae** (Stobbe).
4. ♂: sclerite on tergite 1, if present, without well-developed setae; genitalia with well-developed spines on preputial sac; transverse sclerite on tergite 8 of ♀ entire. **5.**
   ♂: sclerite on tergite 1 with either two or six well-developed setae; genitalia without spines on preputial sac; transverse sclerite on tergite 8 of ♀ either interrupted in the middle or absent. **6.**
5. ♂ without a sclerite on tergite 1; sclerites duplicated on tergites 4 and 5; ♀ large, 1·76 mm. in length. **F. rammei** (Stobbe).
   ♂: sclerite on tergite 1 with two minute setae; sclerites duplicated on tergites 4–6; ♀ smaller, 1·2–1·48 mm. in length. **F. calogalea** (Bedf.).
6. ♂: sclerite on tergite 1 with two well-developed setae; ♀: sclerite on tergite 8 usually present (absent in *F. cynictis*). **7.**
   ♂: sclerite on tergite 1 with six well-developed setae; ♀: sclerite on tergite 8 absent. **F. setosa** n. sp.
7. Spiracles on tergite 2 larger than those on tergites 3–4 in both sexes; basal plate with sides parallel, except at the apex where it abruptly becomes narrower. **F. acutirostris** (Stobbe).
   Spiracles on tergites 2–4 equal in size in both sexes. **8.**
8. ♂: sclerite absent on tergite 6; basal plate V-shaped; ♀: sclerite on tergite 8 absent. **F. cynictis** (Bedf.).
   ♂: sclerite present on tergite 6; basal plate narrower in front than behind; ♀: sclerite on tergite 8 present. **9.**

9. ♂: tergite 8 with two separate median longitudinal sclerites; ♀: gonopophyses as in Fig. 1 *G*. **F. subrostrata** (Nitzsch).
♂: sclerites on tergite 8 united in front; ♀: gonopophyses similar to Fig. 1 *F*. **F. rostrata** n.sp.

10. Gonopophyses of ♀ as in Fig. 1 *B*. **F. helogale** n.sp.
Gonopophyses of ♀ similar to Fig. 1 *F*. **F. genetta** (Bedf.).

F. mungos (Stobbe) and *F. acuticeps* (Neumann) are not included in the above table as it is not known how many abdominal spiracles they possess. The latter is probably closely related to *F. genetta* (Bedf.).

1. **Felicola caffra** (Bedford).

1919. *Trichodectes caffra* Bedford, p. 724, pl. 3, figs. 10, 11.

Described from a female and a male taken off a Cape wild cat (*Felis ocreata caffra* Desm.), at Blockspruit, Rustenburg District, Transvaal. I here designate the male as the holotype. The head is very similar to that of *F. subrostrata* and other closely related species parasitic on mongooses. On each side of the male genitalia there is a narrow longitudinal sclerite.

2. **Felicola viverriculae** (Stobbe).

1913. *Trichodectes viverriculae* Stobbe, p. 375, fig. 4.

Described from specimens taken off a civet cat, *Viverricula rasse*, in Madagascar. I here designate the male the holotype, no previous designation having been given.

Length of male 1·4 mm.; of female 1·5 mm. The lateral margins of the forehead are convex, and the abdomen of the male resembles that of *F. rammei* in shape.

3. **Felicola rammei** (Stobbe).
(Fig. 3.)

1913. *Trichodectes rammei* Stobbe, p. 377, fig. 6.

Described from specimens (both sexes) taken off water mongoose, *Atilax paludinosus* G. Cuv. (= *Herpestes galera*), Tanganyika Territory. Ferris (1930) records numerous specimens, which he considers to be this species, from *Galerella brunneo-ochracea* Matschix, Belgian Congo, and specimens which I regard as probably this species have been taken off Cape Ichneumon (*Herpestes caffer* Gmelin) at Pietermaritzburg, Natal (coll. L. Hill). The type specimens may have been stragglers, or the host misidentified, as Stobbe records a second species from *A. paludinosus*, and this is the only one I have found on this animal.

I here designate the male the holotype, as no previous designation has been given.

Female: the gonopophyses are shown in Fig. 6; the transverse sclerite on tergite 8 is entire.

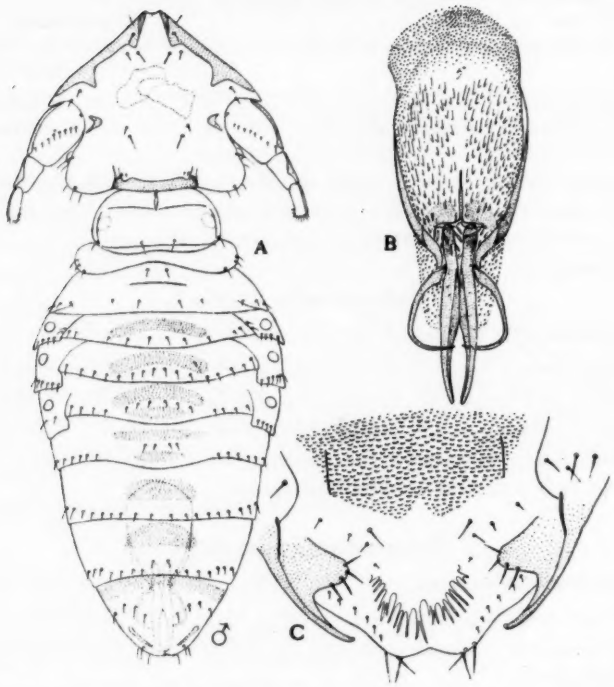

Fig. 3. *Felicola rammei* (Stobbe). *A*, dorsal aspect of ♂; *B*, genitalia of ♂; *C*, gonopophyses of ♀.

4. **Felicola calogalea** (Bedford).
(Fig. 4.)

1928. *Trichodectes calogalea* Bedford, p. 843, pl. 2, fig. 4; pl. 3, fig. 7.

Described from females and males taken off slender mongoose, *Myonax cauui* (= *Calogale cauui*) in the Rustenburg District, Transvaal, and from *M. pulverulentus* (= *C. pulverulentus*), Kenkelbosch, C.P. Specimens have also been taken off *Myonax nigratus* (Thomas), Otjitundua, Kaokoveld, South-West Africa.

Female: the gonopophyses are similar to those of *F. rammei* (Fig. 3*C*); the transverse sclerite on tergite 8 is not interrupted in the middle.

5. **Felicola acutirostris** (Stobbe).
(Fig. 5.)

1913. *Trichodectes acutirostris* Stobbe, p. 378, fig. 7.

Described from specimens taken off water mongoose, *Atilax paludinosus* G. Cuv. (= *Herpestes galera*), Pemba. Numerous specimens have also been taken off the same host, Vredendal, Olifants River (Transvaal Mus. No. 6191).

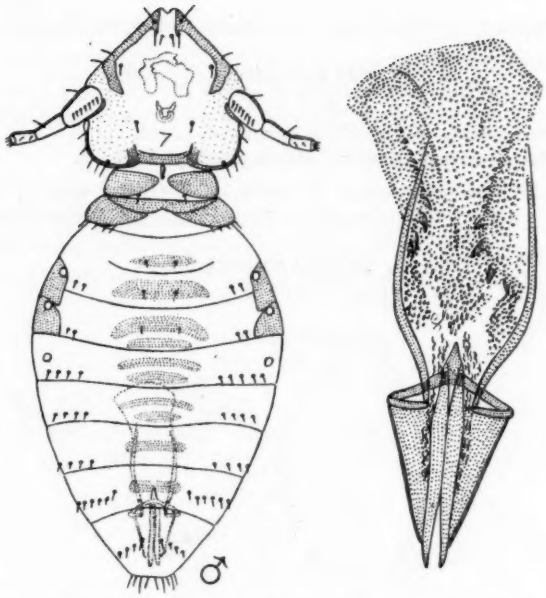

Fig. 4. *Felicola calogalea* (Bedford), dorsal aspect and genitalia of ♂.

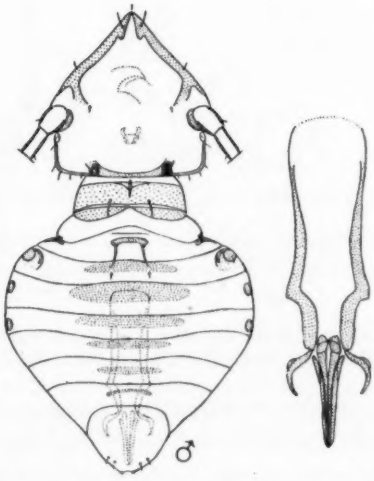

Fig. 5. *Felicola acutirostris* (Stobbe), dorsal aspect and genitalia of ♂.

I here designate the male the holotype, no previous designation having been given.

The gonopophyses of the female are similar to those of *F. setosa* (Fig. 1 *F*).

6. **Felicola subrostrata** (Nitzsch).

(Figs. 1 *G*; 6 *B*, *b*.)

1838. *Trichodectes subrostratus* Nitzsch, in Burmeister, p. 436.
1880. *Trichodectes subrostratus* (Nitzsch) Piaget, p. 389, pl. 31, fig. 9.

This species has been recorded taken off domestic cats in Europe and America; also from a domestic cat at Pietermaritzburg, Natal, but it is not common.

7. **Felicola rostrata** n.sp.

(Figs. 6 *A*; 6 *B*, *a*; 6 *C*.)

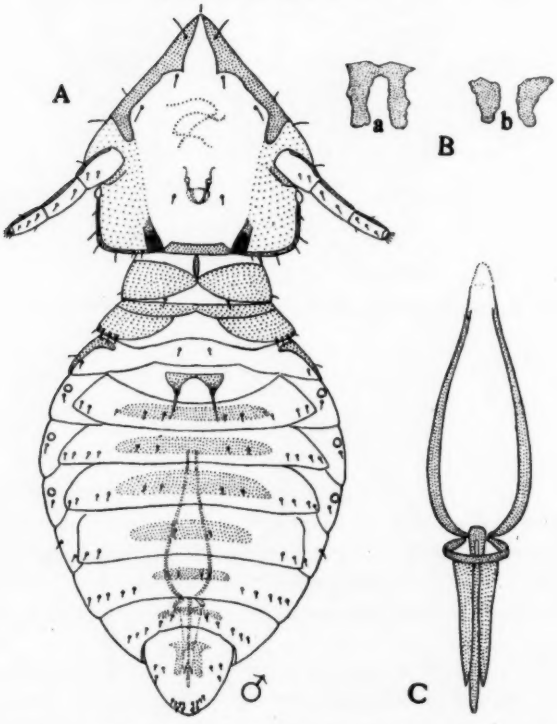

Fig. 6. *Felicola rostrata* n.sp. ♂: *A*, dorsal aspect; *B a*, sclerite of eighth tergite; *C*, *Felicola subrostrata* (Nitzsch) ♂, genitalia. *B b*, sclerites of eighth tergite.

Numerous specimens taken off white-tailed mongoose (*Ichneumia albicauda* G. Cuvier), Umfolosi River, Zululand, 1. vii. 1922 (Transvaal Mus. No. 3003). Holotype a male.

Male. Length 1·03 mm. Head 0·35 × 0·31 mm. Only differs from the

male of *F. subrostrata* in having the median, longitudinal sclerites on tergite 8 united in front.

Female. Length 1·31 mm. Head 0·42 × 0·41 mm. Indistinguishable from the female of *F. subrostrata*, except that the gonopophyses resemble those of *F. setosa* (Fig. 1 *F*). The length of the latter is 1·17–1·29 mm. and the head measures 0·33 × 0·33 mm. to 0·40 × 0·31 mm.

8. **Felicola cynictis** (Bedford).

(Fig. 7.)

1928. *Trichodectes cynictis* Bedford, p. 844, pl. 3, fig. 8.

Described from males and females taken off yellow mongoose (*Cynictis penicillata* G. Cuvier) at Onderstepoort, Transvaal, and at Bothaville, Orange Free State.

9. **Felicola setosa** n.sp.

(Figs. 1 *F*, 8.)

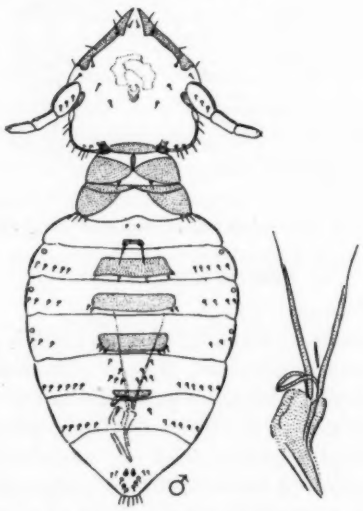

Fig. 7. *Felicola cynictis* (Bedford), dorsal aspect and genitalia of ♂.

Several males and females taken off Selous' mongoose (*Paracynictis selousi* de Winton, Mokeetsi, Transvaal (Transvaal Mus., No. 6201).

Holotype a male.

Male. Length 0·94 mm. Head 0·33 × 0·29 mm. The sclerite on tergite 1 has six well-developed setae; on tergite 5 the sclerite is duplicated; on tergite 6 it is triplicated, and on tergite 7 it is interrupted in the middle. The genitalia are distinct, but resemble those of *F. cynictis* in having the basal plate V-shaped.

Female. Length 1·13 mm. Head 0·35 × 0·36 mm. The gonopophyses are shown in Fig. 1 *F*. Sclerite on tergite 8 absent. It is indistinguishable from *F. cynictis*.

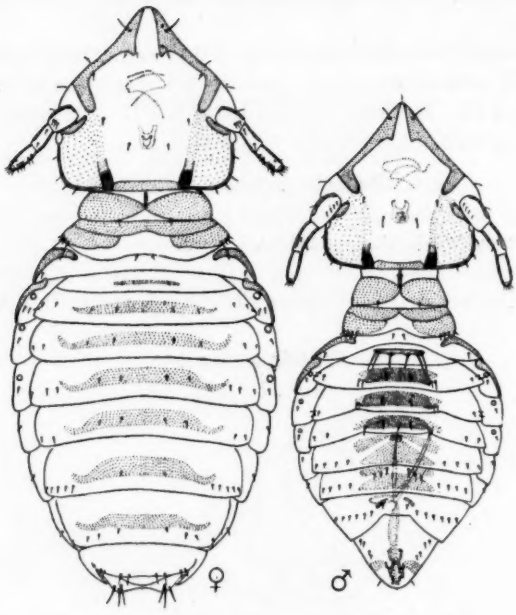

Fig. 8. *Felicola setosa* n.sp., dorsal aspect of ♂ and ♀.

10. **Felicola mungos** (Stobbe).

1913. *Felicola mungos* Stobbe, p. 375, fig. 5.

Described from specimens taken off *Herpestes affinis gracilis*, Tanganyika Territory, and from ruddy mongoose, *Myonax ratlamuchi* A. Smith (= *Herpestes badius*), Zanzibar. The former is probably its true host, and the latter record may have been due to the specimens being stragglers or, as is more likely, the host was incorrectly identified. *F. calogalea* is probably the only species parasitic on species of *Myonax*. I here designated the male the holotype, no previous designation having been given.

This is a small species resembling *F. helogale* in the shape of the head, except that the lateral margins of the forehead are not convex on each side of the middle.

Male. Length 0·92 mm. Head 0·29 × 0·38 mm.

Female. Length 1·15 mm. Head 0·3 × 0·42 mm.

11. **Felicola helogale** n.sp.
(Figs. 1 *D*, 9.)

Two females and one immature male taken off pigmy mongoose, *Helogale parvula brunnula*. Thomas and Schwann, N'jelele River, Northern Transvaal, 6. viii. 1929 (Transvaal Mus., No. 5869). Holotype a female.

This species is probably closely related to *F. mungos*. The female can be distinguished from that of *F. setosa* (Fig. 8) and other related species in the shape of the head (Fig. 9) and gonopophyses (Fig. 1 *D*), and the absence of abdominal spiracles. The sclerite on the anterior margin of tergite 8 is entire. Length 0·87 mm. Head 0·26 × 0·31 mm. The head of the immature male is similar to that of the female, and the abdomen resembles that of *F. acutirostris* in shape.

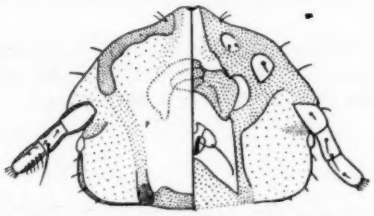

Fig. 9. *Felicola helogale* n.sp., head of ♀.

12. **Felicola acuticeps** (Neumann).

1902. *Trichodectes acuticeps* Neumann, p. 601.

Described from specimens taken off a genet in Abyssinia. I here designate the male as the holotype, no previous designation having been given. Neumann did not figure the species or describe the male genitalia, but it appears to be closely related to *F. genetta*. The first antennal segment of the male is as long as the two succeeding segments together, whereas in *F. genetta* it is shorter than the two last segments.

13. **Felicola genetta** (Bedford).

1919. *Trichodectes genetta* Bedford, p. 725, pl. 4, figs. 12–13.

Described from two slightly immature females and one male taken off small-spotted genet, *Genetta felina ludia* Thomas and Schwann, Jericho, Transvaal. I here designate the male the holotype. Females, which I take to be the same, have been collected from a large-spotted genet, *Genetta tigrina* Schreber, Pietermaritzburg, Natal (coll. L. Hill).

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THE HATCHING ORGAN OF *LIPEURUS COLUMBAE* LINN. (MALLOPHAGA), WITH A NOTE ON ITS PHYLOGENETIC SIGNIFICANCE.

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(With 1 Figure in the Text.)

In a recent paper on the mechanism of hatching in insects (Sikes and Wigglesworth, 1931) a type of hatching organ was described in certain Siphunculata[1] which was quite unlike any that had been observed in other insects. It was of interest, therefore, to compare with this the hatching spines of Mallophaga; for although Voss (1921) records the presence of these organs in Mallophaga, no description of them seems to have been published.

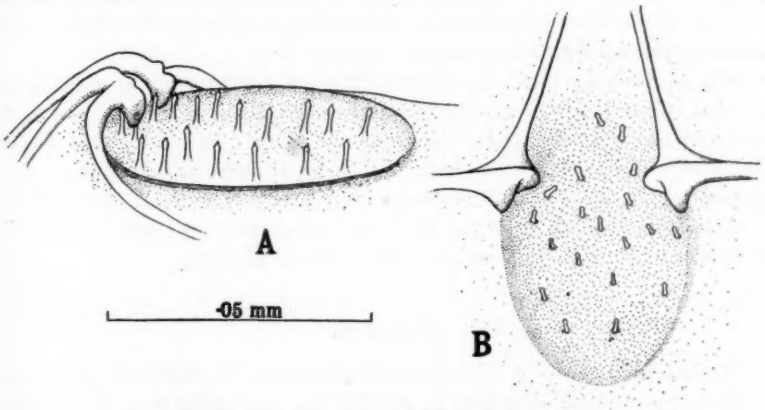

Fig. 1. A, lateral view of hatching organ; B, dorsal view.

The organ has been found without difficulty in the fully developed eggs of the pigeon louse (*Lipeurus columbae* Linn.), and is shown in plan and side view in Fig. 1. It is a specialised part of the pre-larval skin that is shed at the time of hatching, and consists of a thickened plate bearing about 18 elongated spines or blades with lance-shaped points. These arise from a saucer-like depression, below and in front of which is a pair of thick blunt teeth projecting upwards. The general plan of the organ is identical with that already described in the sucking lice.

[1] This organ, as it occurs in *Pediculus*, was first described and figured by Alessandrini (1919).

Discussion.

It is well known that insects belonging to the same or related families frequently possess hatching spines of like form. Thus, the Pentatomidae and Coreidae have a single pyramidal spine on the pre-larval skin; the Reduviidae and Cimicidae have a row of minute teeth on either side of the head, again on the pre-larval skin; the Carabidae and Dytiscidae have a spine on each side of the head, which persists during the first larval instar; the Coccinellidae have teeth on the three thoracic segments, which persist until the first larval moult, and the Chrysomelidae have similar teeth on two thoracic and one abdominal segment; there is a persistent median tooth in the first stage larvae of most if not all Diptera Nematocera (perhaps the almost identical structure in the Siphonaptera is an index of relationship); and the teeth are more or less alike in *Sialis*, *Osmylus* and *Chrysopa*[1].

It is possible, therefore, that the form of the hatching spines might prove of value in cases of doubtful phylogeny.

The systematic position of the Mallophaga has been reviewed by Nuttall (1919), who shows that the whole trend of recent research supports the opinion of the earlier authors in placing the Mallophaga and Siphunculata close together in the system. The form of the hatching spines, as described in the present paper, affords further support for this view.

It is probably too much to expect evidence of more remote ancestry in the hatching spines. Strindberg (1916) considers the Mallophaga to be related to the Isoptera. I have examined the eggs and newly hatched larvae of two species of termites, but have been unable to discover any hatching spine. Mjöberg (1910) derives the Mallophaga from ancestral Psocidae. The existing Psocidae have a hatching organ like a file or saw (Peyerimhoff, 1901; Huie, 1916; Weber, 1931) and altogether unlike that in *Lipeurus*.

Summary.

The hatching organ of *Lipeurus columbae* Linn. is described. It is very similar to that already described in sucking lice, and unlike that of any other group of insects. This resemblance is further evidence for the affinity of the Mallophaga and Siphunculata.

[1] References to original sources will be found in the papers of van Emden (1925), Heymons (1926) and Sikes and Wigglesworth (1931).

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ON THE DISTRIBUTION OF AIR IN THE OESOPHAGEAL DIVERTICULA AND INTESTINE OF MOSQUITOES.

ITS RELATION TO EMERGENCE, FEEDING AND HYPOPYGIAL ROTATION.

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From the British Mosquito Control Institute, Hayling Island.

(With 8 Figures in the Text.)

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INTRODUCTION

The three oesophageal diverticula in mosquitoes (two dorsal and one ventral to the alimentary canal), were observed by Dufour in 1851. He called the ventral one "panse" (paunch), and stated that when the insect's stomach was distended with blood, the paunch contained a colourless or faintly amber-tinted liquid. This he regarded as lymph derived from the cutaneous tissue of the mosquito's victim, which had to be sucked up before the flow of blood through the capillaries could proceed. Grassi (1901) regarded the dorsal diverticula as suctorial organs ("succhiatori accessori") and the ventral diverticulum as either a sucking stomach or crop ("stomaco succhiatore o ingluvie"). He found blood in them immediately after the mosquito had bitten. Giles (1902) used the terms "aspiratory vesicles" and "pneumatic sacs," maintaining that these organs were connected, not with the stomach, but with the tracheal system. He suggested that they might possibly correspond to the air sacs in birds.

Nuttall and Shipley (1903, pp. 173–80, Pl. VI, figs. 1, 4, 7) criticised the views of Grassi and Giles. They investigated the sacs (which they termed oesophageal diverticula and, because of their function and structure, "food reservoirs") and showed (*a*) that the ventral diverticulum of an unfed mosquito contains air 48 hours after emergence from the pupa, (*b*) that the three diverticula are frequently distended with air bubbles and (*c*) that when a mosquito is fed with sugar and serum, either coloured or not, this food entered the diverticula and passed on to intestine subsequently, as shown by the suspended carmine it contained. With regard to the air bubbles observed in the diverticula these authors wrote (p. 177) as follows: "The bubbles are not yet explained. They must have their origin from the outside, that is, they must come in through the mouthparts, either in the process of feeding or afterwards. During feeding air might very well enter if the pumping action of the pharynx, etc., were continued even for a moment after the removal of the proboscis from the fluid which is being ingested.—The small size usually shown by the bubbles argues in favour of their having been carried in through the small oral aperture."

Eysell (1924) called the dorsal diverticula "flight bladders" (Flugblasen) and the ventral diverticulum "store-stomach" (Vorratsmagen). He stated that, during the pupal stage, the diverticula remain empty, but that in the newly emerged imago they are, on the contrary, dilated with air. On a later occasion, Eysell suggested that air swallowed by the imago during emergence eventually passes out through the anus. He obviously still considered that this air lodges temporarily in the diverticula, since he added (on the authority of Schaudinn (1904)) that it is subsequently replaced by carbon dioxide produced *in situ* by the action of a commensal yeast[1].

Patton and Cragg (1913) stated that the "crops" or oesophageal diverticula are usually filled with minute bubbles of gas, but in newly hatched mosquitoes they are collapsed. In mosquitoes killed during the act of feeding they are always found to be full of blood, while a little later (depending on the rate of digestion and therefore on temperature) the blood is almost entirely confined to the mid-gut.

MacGregor (1930) advanced the view that the diverticula act in a dual capacity, *i.e.* as food reservoirs and "air separators"; their latter function being to prevent the entry into the stomach of bubbles arising from "air-locks" occurring in the labrum-epipharynx during feeding. He also stated (1931) that the diverticula of mosquitoes (*a*) within the puparia, or (*b*) after emergence before the insect has attempted to aspirate fluids, are always devoid of air.

Philip (1930) stated that in mosquitoes killed and dissected not less than ten minutes after a blood meal, only traces of blood corpuscles were found in

[1] This much-discussed view of Schaudinn's was controverted by Hecht in 1928. The investigations herewith described show definitely that the diverticula become filled with air and not with carbon dioxide.

the diverticula; but that in mosquitoes interrupted during the feeding there was sufficient blood in the diverticula to give a perceptible colour.

THE AUTHORS' OBSERVATIONS.

I. Material and methods.

The experiments herein described were conducted in our laboratory in April–November, 1931, with the object of discovering which of the conflicting views as to the function of the oesophageal diverticula accord with the facts. The mosquitoes, over 800 in number, were dissected, both before and after their emergence from the pupal skin, under various conditions hereinafter specified. Excepting the "wild" and the hibernating mosquitoes, all insects employed were reared from larvae locally collected, the species being *Anopheles claviger, maculipennis*; *Aëdes caspius, maculatus, rusticus, detritus, punctor, geniculatus, cinereus*; *Theobaldia annulata, subochrea, morsitans, fumipennis, litorea* and *Culex pipiens*. Some dissections of other aquatic diptera (*Mochlonyx velutinus, Chironomus dorsalis, Tanypus nebulosus* and two other Chironomids) were also made.

The following procedure was employed throughout. Pupae were killed with alcohol; they were then transferred into saline solution and dissected forthwith. Imagines on emergence were either treated in the same way, or were at once transferred into the upper vessel of a Moscon incubator—access to the lower vessel, in which water was placed to maintain a moist atmosphere, being prevented by means of a partition of muslin. The imagines were kept thus in readiness either for dissection at pre-arranged times, or for feeding prior to dissection. When required, each imago was chloroformed, moistened with alcohol and immediately dissected—experience having shown that the diverticula were liable to lose some of the contained air if the specimen was kept in fluid for even an hour. Each dissection was effected by careful removal of the body wall and surrounding tissues, tractive forces liable to disturb the contents of the diverticula and mid-gut being thereby reduced to a minimum.

Where it appears necessary the two main portions of the imaginal mid-gut are referred to as the cardia (the anterior narrow part, lying within the thorax) and the stomach (hinder, dilatable part) respectively.

II. On the distribution of air:

(*a*) *In the oesophageal diverticula and mid-gut of mosquitoes before and during the emergence of the imago from the pupal skin.*

The distribution of air beneath the pupal skin prior to, and during, the emergence of the imago was described respectively by Hurst, in 1890, and by Nuttall and Shipley, in 1901. In describing the pupa, the former writer refers to this subject as follows:

"The pupa...floats, thorax uppermost, by virtue of a large air cavity lying under the hinder part of the thorax and the anterior part of the abdomen.

This cavity is bounded in front by the legs, at the sides by the wings, and below by the mouthparts. It extends up at each side of the first segment of the abdomen, where it is covered by the halteres, and into this part of the cavity at each side opens a large stigma, held open by the fairly well-developed cuticular lining ('intima'), and guarded near its entrance by numerous spines. These two stigmata belong to the first abdominal segment, and put the air-cavity just described into direct communication with the tracheal system. As already mentioned, I regard this cavity and these stigmata as being mainly, if not exclusively, hydrostatic in function, serving not only to make the pupa float when at rest, but to make it float in a definite position, with the thorax uppermost and the apertures of the siphons at the surface of the water."

Nuttall and Shipley, who were the first to give a detailed account of the emergence of a mosquito (*Anopheles maculipennis*), described this process in these words:

"When transformation is about to take place the pupa remains quietly at the surface of the water. Fine streaks due to the contained air appear upon the dorsum, extending antero-posteriorly between the respiratory trumpets, anteriorly as far as the base of the head. The air at first only extends a short distance posteriorly to the trumpets, but it gradually accumulates along the dorsal surface until it reaches to about the anterior margin of the seventh abdominal segment. Suddenly the pupa, which has become more buoyant, alters its position by extending its abdomen so that it comes to float parallel to the surface of the water, immediately beneath the surface-film to which it becomes attached. This usually lasts two minutes. Usually a minute later the dorsal surface of the thorax protrudes through the dorsal slit in the pupa-skin and projects above the water. The insect's body seems to "grow" without visible effort out of the pupal covering, although a forward bend in the abdomen, near the thorax, indicates that the insect pushes itself out by pressing the abdomen against the pupa-skin. As the abdomen is protruded, the abdominal portion of the pupa-skin gradually fills with air."

The object of our first series of experiments was to ascertain the condition of the oesophageal diverticula and mid-gut both before and during emergence. The dissection of 70 pupae gave the following results:

(i) Two pupae in the act of straightening out, but having no air beneath the skin, contained no air in either the diverticula or mid-gut.

(ii) Five straightened pupae, with some air beneath the skin, had no air in either the diverticula or mid-gut, but had two small bubbles in the oesophagus.

(iii) Of fourteen imagines surrounded by air while still enclosed in the pupal skin, seven had small bubbles (one to several) in the mid-gut and seven had a long bubble in the mid-gut extending to segments 2–4 of abdomen. None of these imagines had air in the diverticula.

(iv) In 49 imagines dissected after the pupal skin was clearly ruptured, fifteen (less than or about $\frac{1}{3}$ emerged) had air extending to segments 3–4, fourteen ($\frac{1}{2}$ emerged) had air extending to segments 4–5, and twenty ($\frac{3}{4}$ to

almost completely emerged) had air extending to segments 5–6. In the almost completely emerged insects the air extended in nearly all cases to segment 6. None of these imagines had any air in the diverticula.

The results of the above dissections, when considered in conjunction with the observations which preceded them, show that the process of emergence takes place in the following manner:

When the straightening of the pupal abdomen signifies that the time for emergence has arrived, the scales of the imago become visible through the pupal skin, and rhythmical movements of the pharyngeal pump commence. The air from the "vesicle" begins to creep towards the head, forming a bubble at the base of the proboscis before spreading generally under the pupal skin. Before the air has entirely surrounded the imago, a portion of it is aspired through the oral aperture and passed into the mid-gut, where it forms a long, narrow bubble reaching as far back as the fourth abdominal segment and having a diameter about half that of the abdomen. At the same time a further straightening of the imago's abdomen occurs (due in part to muscular action and in part to the increase of internal pressure caused by the swallowed air) with the result that the thorax is pushed forward until it ruptures the pupal skin and thereby establishes direct communication between the oral aperture and the external air. Air is now swallowed more rapidly, further extending the abdomen and increasing its rigidity, the legs and abdomen meanwhile making alternate movements to facilitate the act of emergence. By this time the air within the mid-gut has extended back as far as the sixth segment, the wall of the stomach being now very tenuous and in close contact with that of the abdomen. As soon as the fore-legs are entirely (and the mid-legs nearly) withdrawn from the pupal skin, the body of the imago, which has so far maintained

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Legends to Figs. 1–8.

Figs. 1–8. Illustrating the distribution of air in the mosquito's alimentary tract including the oesophageal diverticula during emergence, etc. (schematic).

Fig. 1. Mid-gut and oesophageal diverticula of imago dissected from pupa prior to appearance of air beneath pupal skin. The shading indicates meconial fluid.

Fig. 2. Pupa showing elongate air bubble in mid-gut of enclosed imago just before rupture of pupal skin.

Fig. 3. Mid-gut and oesophageal diverticula immediately after rupture of pupal skin. The diverticula are empty and extended, while the stomach is distended with air: note air bubble in cardia.

Fig. 4. Imago nearly free from pupal skin. Diverticula are still empty and mid-gut distended with air.

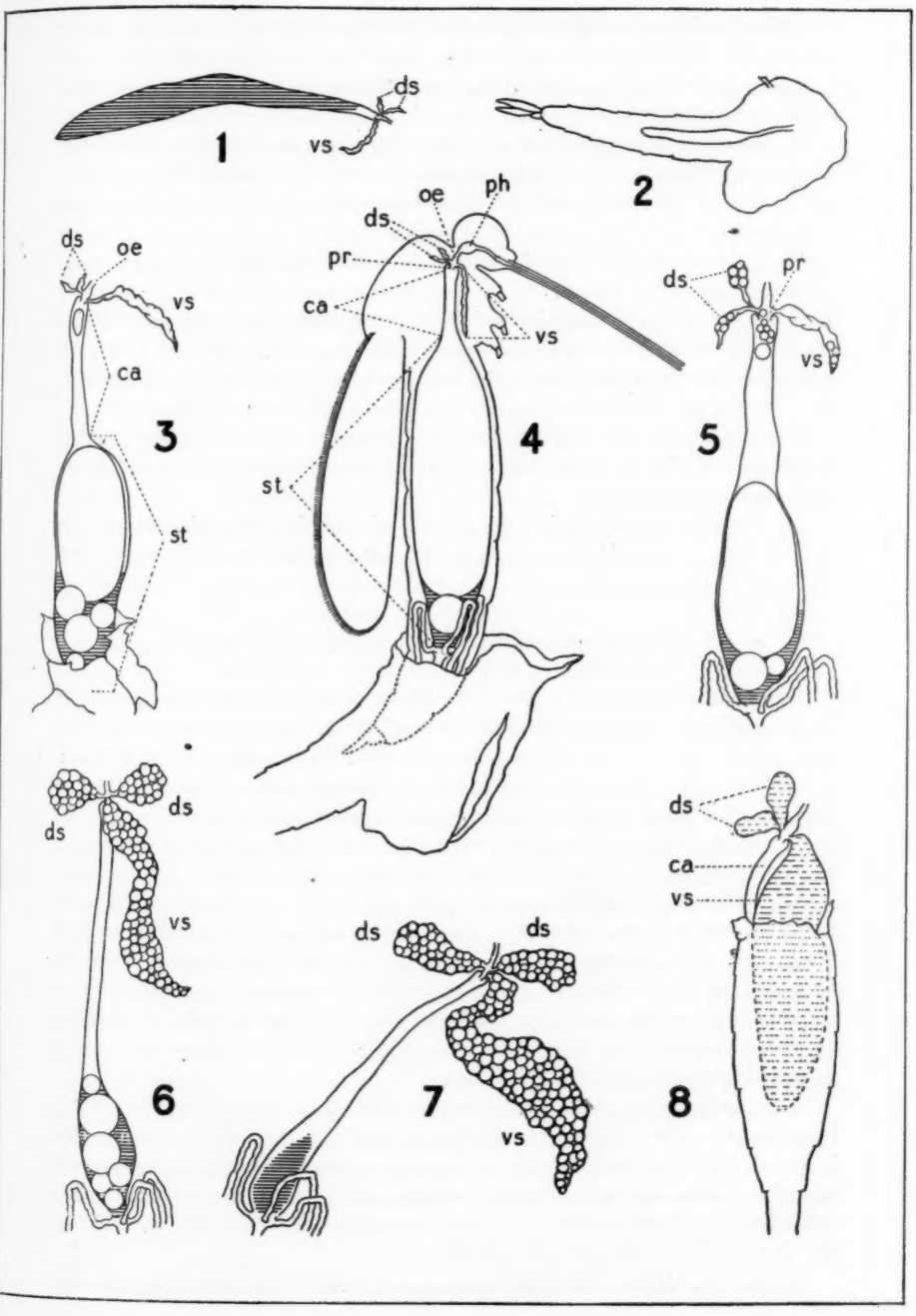
Fig. 5. Mid-gut and oesophageal diverticula 2 hours after emergence. Several bubbles of air in diverticula, also in cardia and proventricular aperture.

Fig. 6. Mid-gut and oesophageal diverticula 5 hours after emergence. Diverticula nearly full of air and mid-gut approaching its final undistended condition.

Fig. 7. Mid-gut and oesophageal diverticula about 15 hours after emergence. Diverticula are full of air and mid-gut empty.

Fig. 8. Imago partly dissected after having access to fruit juice for 5 days. All diverticula distended with fluid, as indicated by shading.

Key to lettering: *ca.* cardia; *ds.* dorsal sac(s); *oe.* oesophagus; *ph.* pharynx; *pr.* proventriculus; *st.* stomach; *vs.* ventral sac.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a vertical position, bends forward until the fore-legs rest upon the water surface. The hind-legs are soon freed in their turn, the fully emerged imago then resting for a brief period, either upon the discarded pupal skin or upon adjacent vegetation.

It should be noted that the air which spreads round the imago (thereby increasing its buoyancy) makes its appearance before the pupal skin ruptures.

The above series of dissections, therefore, enable the following conclusions to be drawn:

(1) The oesophageal diverticula contain no air either prior to, or during, the emergence of the imago.

(2) When the pupa is straightening out prior to emergence its gut contains no air. Air accumulates gradually beneath the pupal skin and small bubbles appear in the oesophagus. When the imago is surrounded with air, being confined within the pupal skin, air bubbles pass from the oesophagus into the cardia and thence to the stomach, the little bubbles fusing to form an elongated bubble conforming to the shape of the dilated stomach and extending to abdominal segments 2–4.

(3) After the pupal skin is ruptured, the air bubble in the mid-gut extends and expands progressively, eventually reaching in most cases to segment 6 when the imago is almost free.

(*b*) *In the oesophageal diverticula and mid-gut of imagines remaining unfed after casting the pupal skin.*

In our second series of experiments 74 imagines were examined, eight of these being dissected immediately after emergence and others after various intervals varying from half an hour up to 22½ hours. The results were as follows:

(i) When the insect emerged and for 1 hour after, the mid-gut contained air extending back to abdominal segment 6, after which the amount of air decreased gradually so that after 7 to say 10 hours it extended only to segment 3 or 1. After 12–22½ hours, 22 out of 28 insects showed no air in the mid-gut.

(ii) Air began to appear in the ventral and dorsal diverticula usually after half an hour (1–3 small bubbles), the amount gradually increasing so that 7–9½ hours after emergence the air in the ventral diverticulum extended to segments 2–4, whilst the dorsal diverticula contained some bubbles or were filled therewith. After 12–22½ hours the air in the ventral diverticulum extended to segments 4–5 in 20 insects out of 28, the dorsal diverticula in all cases being full to half full of bubbles.

The observations made in connection with these dissections show that, immediately after emergence, fluid is passed out through the anus. The abdomen (hitherto much extended and dorso-ventrally flattened) progressively becomes shorter and more circular in cross-section; while the cardia, hitherto dilated with air and pressing upon the ventral diverticulum (and perhaps on the dorsal diverticula) becomes narrower.

About this time a slow contraction of the stomach commences, with the

result that small bubbles of air[1] are gradually expelled forward through the proventriculus into the oesophagus, and thence into the dorsal and ventral diverticula, which have until now remained in an empty, collapsed condition. As this transference of air progresses, the ventral diverticulum (hitherto lying wholly within the thorax) extends backward and becomes distended distally until it occupies four or five segments; while the dorsal diverticula also expand until they fill that part of the thorax (bisected longitudinally by the thoracic muscles) which lies anterior to a cross-section passing through the prothoracic spiracles. The transference of air from the mid-gut to the diverticula requires from 12 to 22 hours for completion.

The experiments show that the air contained in the oesophageal diverticula is sucked in through the oral aperture, as Nuttall and Shipley believed, but it does not pass directly into the diverticula as they supposed; it is first taken into the stomach whence it is afterwards expelled into the diverticula. The diverticula in the newly emerged imago are devoid of air, not dilated with air as Eysell states. It will also be noted that MacGregor's (1930–31) two statements, (1) that air is "separated" by the diverticula to prevent its entry into the stomach and (2) that the diverticula are always devoid of air before the insect has attempted to aspirate fluids, are both incorrect.

Moreover, dissections made on certain other water-breeding diptera, enumerated on p. 370, showed in all cases that swallowed air played a similar part during emergence. The phenomenon deserves further study[2].

(*c*) *In the oesophageal diverticula and cardia of imagines* (A) *that had or had not access to raisins before sucking blood and* (B) *that had access to raisins only.*

A series of dissections was next carried out to determine what happens to the air in the sacs when a female mosquito is given a meal (*a*) of fruit juice, (*b*) of blood and (*c*) of fruit juice followed by blood. These feeding experiments were made with (1) laboratory-reared and (2) "wild" imagines.

The experiments with group (1) were made with imagines which had been isolated without food since their emergence (see p. 370) being either (*a*) placed in a cage with raisins for 2–6 days some being then dissected, and others (*b*) being fed on blood immediately before dissection, (*c*) fed on blood not less than 48 hours after emergence and then immediately dissected.

In the experiments with group (2) the imagines were either (*a*) dissected without being given a blood meal (for comparison group with (1) (*a*) above), (*b*) given a blood meal in the field and dissected within an hour, or (*c*) given a blood meal in the laboratory and immediately dissected.

[1] The bubbles are doubtless formed in the meconial fluid.

[2] Nuttall (1917, pp. 148 *et seq.*) found that the larva of the body-louse, *Pediculus humanus*, swallows air (diffused inwardly through the egg-shell) previous to hatching; the resulting distension of the larva facilitating its exit from the egg. More recently, Sikes and Wigglesworth (1931) have stated that, in the mouse-louse, *Polyplax serrata*, and bed-bug, *Cimex lectularius*, air swallowed by the larva plays a more or less similar part during emergence.

The results of these experiments, which consisted of 303 dissections, are summarised below:

A. *Imagines having had or not having had access to raisins before sucking blood.*

(1). Laboratory-bred imagines (123 dissections):

(i) Of 52 imagines having air bubbles only in the diverticula (seven being tinged with blood) 22 had air bubbles in cardia.

(ii) Of nine imagines having their diverticula less than half full of fluid (one being tinged with blood) two had air bubbles in cardia.

(iii) Of 55 imagines having their diverticula more than half full of fluid (three being tinged with blood) seven had air in cardia.

(iv) Seven imagines having their diverticula full of fluid (one being tinged with blood) had no air in cardia.

(2). "Wild" imagines (38 dissections):

(i) Of 33 imagines having air bubbles only in the diverticula (four being tinged with blood) 21 had air bubbles in cardia.

(ii) Four imagines having their diverticula less than half full of fluid (none tinged with blood) had no air in cardia.

(iii) One imago having its diverticula more than half full of fluid (untinged with blood) had no air in cardia.

B. *Imagines having had access to raisins only.*

(1). Laboratory-bred imagines (89 dissections):

(i) Of 18 imagines having air bubbles only in the diverticula, 11 had air bubbles in cardia.

(ii) Of 13 imagines having their diverticula less than half full of fluid, three had air bubbles in cardia.

(iii) Of 49 imagines having their diverticula more than half full of fluid 10 had air bubbles in cardia.

(iv) Of nine imagines having their diverticula quite full of fluid, one had air bubbles in cardia.

(2). "Wild" imagines (53 dissections):

(i) Of 48 imagines having air bubbles only in the diverticula 30 had air bubbles in cardia.

(ii) Of five imagines having their diverticula less than half full of fluid, one had air bubbles in cardia.

Of 161 imagines which had a blood meal before dissection, 85 (52 laboratory-bred, 33 caught in the open) showed no fluid in the oesophageal diverticula. Some of the laboratory-bred imagines had no access to fluid after emergence; while others were not allowed time in which to feed on raisin juice and void it before dissection. In the case of 33 "wild" imagines it was, of course, impossible to say whether the absence of fluid from the diverticula indicated that they had fasted or fed and voided excreta previously.

In 16 cases only were minute "traces of blood" (generally but a faint

coloration) visible in the diverticula under low power; 11 of these were recorded in cases where the mosquito had had (or had retained) no previous meal.

To test Philip's statement that blood is invariably found in the diverticula if a mosquito is interrupted during feeding, 25 imagines were chloroformed while in the act of biting. In only four of these was any blood coloration perceptible in the diverticula under low power, but examination of the remainder under high power showed the pressure of blood corpuscles in 13 other cases.

Fourteen imagines were also dissected after completing a blood meal—one after an interval of 5 min. and the remainder after intervals of 10–20 min. In two cases only the bubbles in the diverticula were visibly coloured, but under high power corpuscles were found in 11 other cases. This group of experiments shows, therefore, that if a mosquito is fed upon blood (whether previously satiated with fruit juice or not) the blood goes entirely (or almost entirely) into the stomach. This agrees with statements made by previous observers.

It will be noted that, both in the case of fruit-fed and blood-fed mosquitoes, about one-third of the imagines were found to have air in the cardia. In most of these cases only a few small bubbles of air were present, and it is suggested that this air is sucked back (from the lumen of the labrum-epipharynx, buccal cavity, pharynx and oesophagus) immediately in advance of the fluid.

A further series of feeding experiments was made, in which 45 females of *Culex pipiens* were given access to raisins for a period of 5 days. At the end of this time, 18 of the imagines were dissected, while the remaining 27 were kept without food or water for an additional period of 4 days before dissection. The results of these experiments were as follows:

(i) Of the 18 imagines dissected after feeding, only one had an air bubble in the cardia; the ventral diverticulum was full of fluid in nine, and filled with fluid plus 1–8 air bubbles in nine; the dorsal diverticula in six were full of fluid, in 11 they contained fluid and 1–11 air bubbles, and in one they contained fluid and air in equal amount.

(ii) Of the 27 imagines dissected after fasting 4 days, 15 had 1–10 air bubbles in the cardia; the ventral diverticulum contained air in all; the dorsal diverticula in 26 contained only air, whilst it was empty in one case only.

As regards the first group of dissections, it will be noted that, in every case, all three diverticula were found to be full, or nearly full, of fluid (fruit juice): whereas the diverticula of the mosquitoes which had been fed and subsequently starved were without exception devoid of fluid. Of the 27 imagines composing the second group, air was found in the cardia in 15 cases, suggesting that the air which replaces the fluid in the diverticula reaches them in the manner already described in Section II (*b*) (p. 375).

In addition to the experiments with *Culex pipiens* just described, a number of imagines of the coastal mosquito *Aëdes detritus* were similarly dissected after consecutive periods of feeding and starvation. As the results obtained were practically identical with those already mentioned it has not been thought

necessary to tabulate them. They indicate, however, that the data above considered are in no way specially characteristic of hibernating mosquitoes.

We see, therefore, that a meal of blood goes direct into the mid-gut of a mosquito, but that one of fruit juice goes into the oesophageal sacs. The "switching mechanism" still remains to be explained. Christophers considered that the proventriculus and the posterior opening of the pharynx met each other half way, and thus "shut off" the "large oesophageal diverticulum" (i.e. the ventral one), but he seems to have ignored the dorsal diverticula. It appears to us—having regard to the close proximity of the proventriculus to the entrance to the three oesophageal diverticula—that any relaxation of the annular muscle of the proventriculus must necessarily tend to interrupt communication between the oesophagus and the diverticula—ventral and dorsal equally. We also suggest that the accumulation of swallowed air in the mid-gut of the imago during emergence, and its contemporaneous absence from the diverticula, may most probably be explained in a similar manner.

(*d*) *In the oesophageal diverticula of* Culex pipiens *prior to, and during, hibernation.*

In this series of observations 169 females of *Culex pipiens*, collected in two dark sheds, were dissected. Sixty-two of these were collected during October and November, 1931; 55 on February 19th, 1932; and 52 on March 23rd, 1932.

As regards the diverticula of the imagines dissected in 1931, eight contained air and fluid in varying proportions, and the remainder contained air only—15 of these being more than half full, 35 not more than half full and four entirely collapsed.

Of the 55 imagines collected in February, the diverticula were in all cases devoid of fluid. The ventral diverticulum was collapsed in one case, not more than half full of air in 41 cases, and more than half full of air in 13 cases. The dorsal diverticula were collapsed in two cases, not more than half full of air in 35 cases, and more than half full of air in 18 cases.

Of the 52 imagines collected in March, the diverticula were also in all cases devoid of fluid. The ventral diverticulum was not more than half full of air in 30 cases, and more than half full of air in 22 cases. The dorsal diverticula were not more than half full of air in 21 cases and more than half full of air in 31 cases. Six of the imagines had air bubbles in the cardia.

The above observations suggest that, when a mosquito hibernates, the air in the diverticula becomes reduced in quantity (sometimes disappearing altogether) prior to, and increases in amount towards the end of, the hibernating period.

(*e*) *In the oesophageal diverticula and mid-gut of mosquitoes in relation to hypopygial rotation.*

In 1902, Snodgrass pointed out that in males of the Asilid genera *Dascillis* and *Laphris*, the hypopygium is twisted through an angle of 180°. This hypopygial rotation has since been met in many other families of insects, its

occurrence in mosquitoes being first mentioned by Christophers (1901), who stated that the rotation occurs shortly after the emergence of the imago. No subsequent attempts, however, seem to have been made to determine precisely when rotation takes place.

Having observed in the course of a second series of dissections that the hypopygial rotation and the transference of air from the mid-gut to the diverticula took about the same time to complete, dissections of 54 male imagines were made at various intervals following emergence.

We found that rotation to 180° was only effected in males (9) in which there was no air in the mid-gut, the ventral diverticulum containing air extending backward to the 5th abdominal segment. In one male, in a similar condition to the foregoing, rotation to 135° had occurred. Of 19 males which showed a rotation of about 90°, 15 were in a similar condition to the foregoing except that the air in the ventral diverticulum extended to the 5th, 4th, 3rd or 2nd segment, leaving four males with some air (a small bubble, or air extending to segment 2 or 3) in the mid-gut and air (extending to segment 4, 2 or 1) in the ventral diverticulum. Of five insects showing about 45° rotation, four had no air in the mid-gut and air extending to segment 4 or 3 in the ventral diverticulum, and one insect had air extending to segment 2 in the mid-gut and to segment 1 in the ventral diverticulum. In 20 insects in which rotation did not appear to have started there was usually air in the mid-gut as also in the ventral diverticulum in moderate amount.

Conclusions. The foregoing observations show that the hypopygial rotation to 180° reaches completion when the air in the insect's mid-gut has been expelled into the oesophageal diverticula. Why this transfer of air to the diverticula coincides with the rotation being effected remains to be explained.

III. NOTE ON MOSQUITO BITES.

Some observations on the effect of mosquito bites may be of interest. Imagines under 24 hours of age appeared unable to penetrate the skin, owing to the proboscis bending about in the attempt, owing to insufficient hardening. The bite of an imago 24–28 hours old appeared to cause less irritation than that of an older mosquito. Generally speaking, the bites of the Aëdine mosquitoes used in the experiments (*Aëdes caspius*, *rusticus*, *detritus*, *punctor*, *maculatus* and *geniculatus*), produced greater irritation than those of *Anopheles claviger*, *A. maculipennis*, *Theobaldia annulata* or *Culex pipiens*: the last-mentioned species was induced to bite only very rarely. It was noticed that a mosquito might penetrate the skin at as many as eight different points, producing that number of wheals, but not drawing blood. Keeping a mosquito without food from the time of emergence or giving it an unlimited diet of fruit juice seemed to make no difference to the effect of its bites. Two "wild" imagines of *Aëdes detritus*, dissected after a blood meal, were found to have a large number of yeast cells in their sacs, but no unusual reaction to the bites was experienced.

IV. Summary.

1. Shortly before the imago emerges, air accumulates under the pupal skin. This air is swallowed by the imago and passes directly into the stomach; the resulting distention of the abdomen helping to push the thorax forward so as to rupture the pupal skin.

2. After rupture of the pupal skin, the imago continues to swallow air, which causes the stomach (and therefore the abdomen) to expand further and thereby facilitate the emergence of the imago.

3. Within an hour after complete emergence of the imago, the air in the mid-gut commences to pass forward into the (hitherto empty) oesophageal diverticula, and after 12–22 hours the mid-gut no longer contains air. (*Note.* We find that the process of emergence here described for Culicidae also applies to some other diptera which, like mosquitoes, live for a part of their lives in water.)

4. In mosquitoes fed on liquid (*e.g.* raisin juice) other than blood, the liquid goes direct into the oesophageal diverticula and displaces the air from them more or less completely (the experiments of Nuttall and Shipley (1903, p. 177) showed the air to be thus displaced, but this point was not specially mentioned by them).

5. When mosquitoes feed on blood (whether previously fed on raisin juice or not) all the blood goes direct into the mid-gut. (*Note.* Traces of blood, negligible in quantity but sufficient to cause visible coloration, are occasionally to be observed in the diverticula. This appears more likely to happen in cases where no previous meal of raisin juice has been taken.)

6. When a mosquito is fed on raisin juice and subsequently starved for a few days, the fluid contents of the diverticula are entirely replaced by air. The experiments indicate that this air reaches the diverticula via the cardia, in the same manner as during their initial inflation.

7. In the case of "domestic" mosquitoes, the air in the diverticula becomes reduced in quantity (sometimes disappearing altogether) prior to hibernation, and increases in amount towards the end of the hibernating period.

8. In the case of male mosquitoes, the transference of air from the mid-gut to the oesophageal diverticula proceeds *pari passu* with the turning of the hypopygium, the two processes terminating at about the same time.

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THE BIONOMICS OF *IXODES RICINUS* L., THE "SHEEP TICK" OF SCOTLAND.

By J. MACLEOD, B.Sc.

From the Moredun Research Institute, Gilmerton, Edinburgh.

(With 4 Charts and a Map.)

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Introduction.

The work recorded in this paper was carried out at the Moredun Institute as part of an investigation, undertaken by the Animal Diseases Research Association, into the possibility of control of *Ixodes ricinus*, known in Scotland as the "sheep tick." This tick is recognised as a major scourge of farm stock in Britain. In England and Northern Europe it is the vector of a small piroplasm causing red water in cattle. It is a pest of sheep in hill districts, seriously affects their comfort and well-being, and appreciably affects the quantity and quality of the fleece. Recent investigations conducted by the Institute have shown that *I. ricinus* transmits the virus of louping-ill, a widespread disease of sheep. It has also been shown to transmit in sheep the causal agent of a hitherto unrecognised disease, tick-borne fever, which is of considerable importance both because of its debilitating effect upon affected animals, and because its presence may predispose the sheep to the development of serious secondary affections.

Ixodes ricinus L., known as the sheep tick, grass tick, or castor bean tick, commonly affects sheep in Britain. Another tick, *Haemaphysalis punctata* Can. and Fan., which infests sheep in England, has not so far been recorded in Scotland, and has not been observed by us. *Ixodes hexagonus* Leach has been found on sheep in the Ettrick district, but only on rare occasions.

Methods employed for collecting, breeding and handling ticks.

Collection.

For collecting unfed ticks, the "blanket method" was found most satisfactory. Along one edge of an ordinary white woollen blanket a half-inch iron rod or a stout pole was attached, and a long piece of string tied to its ends. The blanket was dragged slowly by the string over the ground from which it was desired to collect ticks. At every 50 yards or so, the blanket was turned over and the ticks adhering to it removed by fine forceps. This method was found very suitable for nymphs and larvae, which become entangled in the woollen fibres, but the loosely adhering adults are liable to be rubbed off.

It is best to put the ticks as they are collected into a bottle containing water[1] in which they sink or are held by surface tension. Ticks suffer no harm from temporary immersion, being very resistant to immersion in water.

On infested pastures the unfed stages may usually be collected with comparative facility in fairly swampy hollows, where there are rushes or rank grass. Dry hillocks where the sheep lie resting, or the vicinity of feeding boxes, often yield a number of ticks. Adults, chiefly males, may be collected from the wool of sheep, especially on a warm sunny day, when the unattached adults roam on the surface of the fleece.

Gorged females can be found during the tick season at the margins of the ears, round the neck at the junction of the wool with the hair of the head, and in the short wool on the outside of the legs. On the bare parts of the body ticks do not remain attached for any length of time after engorgement. Consequently replete females are seldom found there. Gorged nymphs occur on the ears, along the bridge of the nose, and under the lower jaw. Larvae are found attached to the lower parts of the legs, often immediately above the hoof, round the mouth and nostrils, under the jaw, and to a less extent on the face and ears.

The collected material was usually stored in Petri dishes containing some dampened silver sand. It was found necessary to ring the inside of the lid with a strip of plasticine, to prevent the escape of the larvae.

If the water bottle was used for collection, its contents were filtered through coarse filter-paper, and the paper with the ticks then placed in a Petri dish. This method was quite satisfactory and obviated the tedious process of picking out each tick from the water with brush or forceps.

[1] It was found that if moss were used instead of water the ticks very soon gathered under the cork, and the larvae, especially, swarmed over the lip of the bottle each time the cork was removed.

Rearing.

The various stages were fed on hedgehogs or on sheep. With hedgehogs the method used was that recommended by Langeron (1926, *Précis de Microscopie*, 4th ed., p. 753). The ticks are placed in a muslin bag, into which the hedgehog is dropped, and the bag tied with a draw-tape. The hedgehog is left in this for a few hours, and then removed. When the ticks are ready to drop off, the hedgehog is placed in a cage with a perforated bottom, and the cage suspended over a water bath. The gorged ticks drop on to the floor of the cage and through the perforations into the water, from the surface of which they can be removed at convenience.

With sheep, the "scrotum sac" proved the only satisfactory method. Ear-sacs of various types were used. With these it was found possible to get fairly satisfactory infestations, but the recovery of the gorged ticks was unsatisfactory, as the sheep were inclined to rub the sacs off the ears, however securely they were tied. With tups, however, a scrotum sac permits of ticks being gorged and collected without danger of their escaping.

Gorged ticks were kept on moistened sand in Petri dishes. The lids were ringed with plasticine to conserve the moisture. Isolated lots of ticks were kept in small tubes containing a little moist sand and plugged with cotton-wool. These tubes were stored in a large vessel upon a pad of cotton-wool saturated with water. The cover of the vessel was sealed with vaseline to insure a moist atmosphere and water was periodically added to compensate for loss by evaporation.

Moulds may grow heavily on the outside of the tubes, but are seldom troublesome inside. (Data labels should be kept inside each tube, otherwise they rapidly become mouldy and indecipherable.)

Observations of previous authors regarding the biology of *I. ricinus*.

The following abbreviations are used for references to authors:

(A.) Ashworth (1909, p. 133); (B.) Bertkau (1881); (K.) Kossel, Schütz, Weber and Miessner (1903); (N. 1 and N. 2) Nuttall (1911 and 1913); (Sa.) Samson (1909); (St. 1 and St. 2) Stockman (1911 and 1916); (W. 1 and W. 2) Wheler (1899 and 1902). The data contained in the foregoing papers are partly cited from Nuttall (1911).

Pre-oviposition period of females.

Days	Temp.	Author
14	—	Sa.
22	—	W. 1
17–24	24° C. (mostly 17th day)	N. 2

Oviposition lasts.

Days	Temp.	Author
32–45	19° C. (2400–3200 eggs laid)	N. 2

Time different stages feed on host.

	Days	Hosts	Author
Larvae	3–6	Cattle, guinea-pig	K.
	3–6	Hedgehog	N. 1
	4–10	Calf	N. 2
Nymphs	3–5	Cattle, guinea-pig	K.
	3–6	Ram (on scrotum)	N. 2
	4–5	Hedgehog	N. 1
	4	?	W. 1
	4–13	?	St. 1
Female	4–7	Ram (on scrotum)	N. 2
	7	Guinea-pig	K.

Time required for metamorphosis.

	Weeks	Season or temp.	Author
Egg to larva	6	—	K.
	7	—	B.
	8	May–July	W.
	7–9	15–20° C.	N. 2
	36	Sept.–June	A.
Larva to nymph	4	Summer	K.
	10	Winter	K.
	12	Winter, in room	N. 1
	18	10° C.	N. 2
Nymph to adult	8	Summer	K.
	8	Shortest period	N 1
	11	Summer	W. 1
	28	Winter	K.
	48	Kept cool	W. 2

Longevity of unfed tick at different stages.

	Months	Remarks	Author
Larvae	5¾	At 12° C. in corked bottle, still lively	N. 2
	10½	In August–June	W. 1
	19	—	W. 2
	36	On damp sand	St. 2
Nymphs	5–7	On damp sand, from September on	N. 1
	18	—	W. 2
Adults	15–27	Survived	W. 2

The time required for metamorphosis is reckoned as follows (Nuttall, 1913, p. 95): (1) *Egg to larva*: from the date on which the female began ovipositing to the day on which the first larvae emerged from the egg-shells; (2) *Larva to nymph*: from the date on which the larvae dropped off gorged to the day on which the first nymphs emerged; (3) *Nymph to adult*: from the date on which the nymphs dropped off gorged to the day on which the first adults emerged.

Life history of *I. ricinus*.

Oviposition.

When the gorged and fertilised female drops from the host to the ground, she seeks shelter and, after a period, proceeds to lay eggs[1]. The eggs are laid

[1] For a description of the process of oviposition see Nuttall (1911, p. 308).

at the roots of grass, and among vegetable débris. Each egg is covered by a viscid secretion which prevents desiccation.

The length of the pre-oviposition period varies with the season, and with the individual. Gorged females, placed in tubes containing a pad of moist filter paper, and kept in the laboratory at 60–70° F., began egg-laying after 14–52 days (usually 15–22 days).

The larva.

The rate of development of the embryo within the egg depends largely on environmental conditions. Thus, eggs laid in May and kept outside on a grass plot, began to hatch in July, *i.e.* after 9–10 weeks. Eggs laid in May and June and kept in the laboratory in summer at 60–70° F. began to hatch in June and July respectively, *i.e.* after 4 weeks. Eggs laid in September, and kept in the laboratory in winter at 50–60° F., began to hatch in May of the following year, *i.e.* after 37 weeks.

Hatching of the larva occurred by a longitudinal rupture of the egg shell along one side. When newly hatched, the larvae were comparatively inactive, and remained for several days near the remains of the egg cluster; they did not readily attach themselves to a passing object, and, if put on human skin, did not feed.

In July, a cluster of eggs in process of hatching was placed at the roots of grass in the open. Eight days later, the larvae were still near the cluster. After 22 days they were wandering on the grass blades, or waiting on the margins, with fore limbs outstretched in the air, in their characteristic posture when waiting for a host. Now they readily attached themselves to a hand brushed against the grass.

The time that elapses before larvae become active varies. Wheler (cited by Nuttall, 1911), in reckoning the time required to complete the life cycle, allowed 10 days for the larva to harden and become active. It took 22 days in our observation noted above, but, on another occasion, larvae which hatched in July attached themselves to a hedgehog after 9 days.

When hardened, the six-legged larva climbs up grass where, in warm weather, it awaits the passing of a host, clinging to the blade of grass by its two pairs of hind legs; the fore-legs, in the tarsus of which is situated the sensory "Organ of Haller"[1], are often observed waving about in the air. Several larvae may be found in this position on the tip of each blade. It was found that immediately an air current was set up, *e.g.* by breathing on the grass, or if the grass were otherwise disturbed, the larva became excitedly alert, and, relaxing its hold with its middle pair of legs, rose on its hind-legs, ready to transfer itself to the disturbing object. This reaching out was so pronounced that several actually overreached themselves and became detached from the leaves.

Once attached to a passing body, the larva moves about carrying its front

[1] See Nuttall, Cooper and Robinson (1908). On the structure of "Haller's Organ" in the Ixodoidea. *Parasitology*, I, 238–42, Pl. 18 and 1 text-fig.

legs upraised and waving in the manner of feelers. Haller's organ, on each front leg, apparently enables the tick to distinguish between animate and inanimate objects. It has been shown for *Argas persicus* larvae (Hindle and Merriman, 1912) that when these organs are removed, the larvae will attach themselves to a membrane stretched over fluid kept at body temperature. In the event of the object to which it has attached itself proving unsuitable, the tick drops off to await another opportunity. If, however, it is put on warm skin, *e.g.* the back of the hand, it wanders about for a time, but now, as if satisfied as to the nature of the object on which it finds itself, it uses all its legs in walking. Its palps continuously explore the surface as if seeking a point for attachment and finally the tick proceeds to bury its mouth-parts in the skin.

The time required for engorgement of the larva appears unaffected by the temperature of the air; with *I. ricinus* it was found to be the same in winter as in summer.

In the following experiments (Table I) four hedgehogs were used as hosts for *I. ricinus* larvae, the muslin bag method (see p. 384) being employed. The

Table I. *Recording the time required for engorgement of larvae.*

	Gorged larvae recovered after						
	68 hr.	72 hr.	88 hr.	96 hr.	120 hr.	144 hr.	Over
Hedgehog I	1	22	—	217	132	5	
„ II	—	15	—	131	45	9	
„ III	—	2	60	160	225	38	7
„ IV	5	10	147	135	—	120	17
Average		12	103	142	151	35	

time required for engorgement and dropping from the host is calculated as from the time when the unfed larvae were placed on the hedgehog.

The sign — indicates that no count was taken for that interval. Most of the larvae were recovered within 120 hours. In the case of hedgehog IV, no 120-hour collection was made, and probably many if not all of the ticks recovered at the next count (144 hours) had dropped off within 120 hours. It appears, then, that larvae mostly complete their engorgement on a hedgehog in 88–120 hours; the shortest period was 67½ hours. Engorgement on the sheep was completed in 65 hours.

When fully gorged the tick relaxes its anchoring mouthparts, but makes no active effort to leave the host; it awaits, passively, to be shaken or rubbed off. Thus it is that when sheep are kept moving in a pen many ticks drop off simultaneously whereas normally these would have continued to drop off over a period of hours, as chance loosened them from the hairs or wool.

The gorged larvae, on being dropped on a grass tuft, crawled away among the roots into chinks and crevices, and in a short time, none could be found without probing for them in the soil.

The nymph.

The nymph emerges from the larval skin through a rupture on the dorsal surface, behind the capitulum. The time elapsing between engorgement of the larva and the emergence of the nymph varied with the season. Gorged larvae were kept in glass tubes in the laboratory and nymphs issued as follows:

Larvae gorged	Nymphs emerged	Interval
16. ix. 29	28. v. 30	37 weeks
8. viii. 30	25. ix. 30	7 ,,
24. viii. 30	3. x. 30	6 ,,
10. x. 30	7. v. 31	30 ,,

The nymph required some days to harden, during which time it remained in the shelter of the surface soil. When hardened, it climbed up on the grass blades, and behaved in the manner described for the fasting larva.

The time required by the nymph to complete engorgement appears fairly constant. Table II records the numbers of gorged nymphs recovered each day from each of five sheep infested:

Table II. *Recording the time required for engorgement of nymphs.*

Date of infestation	Gorged nymphs recovered on			
	4th day	5th day	6th day	7th day
Sheep I 31. vii. 31	87	56	—	3
,, II 13. viii. 31	2	43	11	11
,, III 31. viii. 31	2	27	3	1
,, IV 3. ix. 31	7	62	11	8
,, V 6. ix. 31	28	57	14	7
Average	25	49	10	6

The table shows that the time for engorgement was 4–7 days, mostly 5 days. This is more evident if the record for Sheep I be ignored. The average for the remaining 4 is 10 for the 4th day, 30 for the 5th, 10 for the 6th and 7 for the 7th day.

The gorged nymph behaves like the larva after falling to the ground where it undergoes metamorphosis to the adult state.

Under similar laboratory conditions, with a summer temperature range of 60–70° F., metamorphosis of the nymph takes longer than that of the preceding stage.

Nymphs gorged	Adults emerged	Interval
31. iii. 30	13. vi. 30	10 weeks
31. iii. 30	12. viii. 30	19 ,,
8. v. 30	26. viii. 30	16 ,,

The adult.

The moult occurred by rupture of the nymphal skin along the anterior margin and down each side to near the third coxae. The cuticle weakened along a band across the dorsum between the third pair of legs. The part of the dorsum anterior to this was pushed up like a flap, by the escaping adult.

The adult cuticle requires about a week to harden, and then the sexes are

ready to seek a host. Their behaviour is similar to that described for the fasting larvae.

Attached females at all stages of engorgement were found coupled with males, and in the laboratory the latter readily couples with unfed females even before hardening of the latter is complete. Males were occasionally found lightly attached to the skin of sheep, but they did not remain attached for more than a few hours. Their main function is to seek for and pair with the females. The sexes remain *in copulâ* for several hours. One pair was observed to remain coupled for over 24 hours.

The time required by the female to complete engorgement varies somewhat. Table III records the numbers of gorged females recovered daily from each of four batches of infested sheep.

Table III. *Recording the time required for engorgement of females.*

Date of infestation	Gorged females recovered					
	7th day	8th day	9th day	10th day	11th day	Over 11 days
Batch 1 6. viii. 31	1	1	—	—	1	1, 24 days
„ 2 13. viii. 31	—	2	3	2	—	1, 12 days
„ 3 22. viii. 31	3	12	6	—	2	
„ 4 31. viii. 31	—	1	1	1	—	
Total	4	16	10	3	3	

The female, therefore, usually requires 7–11 days to engorge, but occasionally as long as 24 days are needed; most of the females drop off 8–9 days after attachment.

The gorged and fertilised female, dropping off the host, after a time proceeds to lay eggs, and so the life cycle is recommenced. On completion of egg laying, the spent and shrivelled females die, usually in about a week, although some may survive a month or longer.

Sex ratio in adults.

From several hundred gorged nymphs bred out in the laboratory, there emerged 671 females and 362 males, *i.e.* about a 9: 5 ratio. The possibility of the difference being due to a differential mortality in the sexes, under artificial conditions, is discounted by the fact that practically all the gorged nymphs moulted.

Longevity of unfed stages.

A remarkable feature of ticks in general is their extraordinary fasting power, and *I. ricinus* is no exception to this rule.

Unfed females and males, collected in May, 1929, and kept in the laboratory in a moist atmosphere lived for 21 months; a male was still alive after 31 months. Larvae which had hatched in June, 1930, were able to engorge in September, 1931, *i.e.* after fasting for 15 months. Nymphs which had moulted in July were able to engorge on a sheep, after fasting 13 months.

Probably under natural conditions, when their energy is expended more

rapidly because of changes in weather conditions which may induce sporadic bursts of activity, the ticks do not survive so long as they do under the equable conditions of a laboratory. Even in nature, however, their powers of survival without a meal are remarkably high.

A 10 acre piece of ground on a hill farm in the Ettrick valley was enclosed in May, 1929, with a game-proof fence of woven wire netting with a mesh of less than two inches. The pasture was of the ordinary type for that farm, which was badly infested with ticks. In September, 1930, a blanket was dragged over the herbage in the enclosed area and active males, females and nymphs were found adhering to its under surface; nymphs were collected by the same method in May, 1931.

If one excludes the possibility of their having fed in the interval on birds or small mammals, these ticks must have dropped from their hosts on to the pasture not later than May, 1929, and survived for 24 months, either as fasting nymphs, or as gorged larvae which had moulted in the interim.

Bionomics.

Hosts (mammals and birds).

In Scotland, the sheep is the chief host of *Ixodes ricinus*, but the parasite is found on many other animals. Williams (1883) records that "rabbits, ferrets, stoats, weasels, and more rarely hares are attacked by ticks." Carmichael (1884) "found *Ixodes* in considerable numbers on the bodies of rabbits, hares, and in one case on a roe deer." Nuttall (1911, p. 152 and 1916, p. 331) records as hosts in Scotland sheep, cattle, red deer, dog, wildcat, *Lepus variabilis* and grouse.

The following records are the result of a monthly examination of small mammals and birds during April–August in two successive years (1930–1). They were sent from two localities in the Borders, Newcastleton and Hawick, enclosed in muslin bags, into which they had been dropped immediately on being killed.

The following hosts yielded one or more stages of the tick. The figures in parentheses give the number of hosts examined; the symbols ♂, ♀, o, l stand for ticks; males, females, nymphs and larvae respectively.

Mammals: Fox (4) ♀, o, l; Hedgehog (9) ♀, o, l; Hare (5) o, l; Rabbit (5) ♀, o, l; Stoat (2) l. Ticks were also found on horses, collie dogs and a cat.

Birds: Hoodie-Crow (3) o, l; Jackdaw (4) o, l; Starling (2) o, l; Golden Plover (2) l; Kestrel (2) o, l; Merlin Hawk (1) l; Barn Owl (1) o.

The ticks were mostly found in April–June; none was found in July; a few were found in August.

The identifications of ticks obtained from birds were very carefully made, as it was expected that species other than *I. ricinus* would be found. It may be of interest to record that *I. caledonicus* Nuttall was found in May on three birds (jackdaw, starling, kestrel) sent from the Newcastleton district. The determination was made by Prof. G. H. F. Nuttall.

The foregoing records combined with those of other workers afford the following extensive list of hosts on which the tick may survive in the absence of sheep: horse, cattle, deer, hedgehog, dog, fox, cat, stoat, weasel, ferret, hare, rabbit, rat, squirrel, hoodie-crow, jackdaw, golden plover, barn owl, grouse, kestrel and merlin hawk. Only immature stages were found on birds.

Variations in susceptibility of sheep to infestation.

The susceptibility of a sheep to infestation appears to depend on its physical condition and breed.

(1) *Effect of physical condition of sheep.* In the same herd there is often a marked variation in the liability of individual sheep to infestation, and this appears to be correlated with the condition of the sheep. It is said by shepherds that ewes weakened by lambing are often more heavily infested than the better conditioned barren ewes. Actual figures of the relative infestations of barren and "lambed" ewes have been obtained in one instance. Two Blackface and one Cheviot ewe, running on the same pasture, were examined in May. One Blackface was barren, the other, and the Cheviot, were with lamb.

Tick infestations of barren and lambed ewes compared.

	Females	Nymphs	Larvae
Blackface, barren	7	58	Few
Blackface, with lamb	20	70	Many
Cheviot, with lamb	27	72	Many

"Non-acclimatised" sheep, when first exposed on certain farms, lose condition at first, and remain poor for some time. Chart I shows the average infestation of a number of "non-acclimatised" sheep exposed for the first time on a tick-infested farm, as compared with "acclimatised" native stock. The "non-acclimatised" sheep were put on the farm on April 2nd, and the counts began on the 14th. The native stock showed at first a low degree of infestation, which increased as the tick season advanced. The "non-acclimatised" sheep rapidly lost condition during the first two weeks of their exposure on the farm, their body weight decreased, and they were generally dull and in poor health. When examined on the 14th, they were found to be much more heavily infested than the native stock. As the sheep began to recover condition, the degree of infestations in the two groups approximated, until, by the middle of May, the experimental sheep were no more heavily infested than the native stock. The number of ticks on both groups decreased towards the end of May.

Explanations of the above phenomena offered by shepherds are (1) that poorly thriving sheep graze more closely and collect more ticks, whereas well conditioned sheep graze more superficially and (2) ill-conditioned sheep lie down more often than thriving sheep.

It is probable that the amount of grease in the wool affects the degree of infestation, dry skinned unhealthy sheep offering more favourable conditions to ticks seeking attachment than sheep with a well-nourished, greasy skin and

fleece. A vicious cycle is established; the ticks lower the condition of the sheep, and these, in consequence, become more liable to further infestation.

(2) *Comparative susceptibility of different breeds.* Chart II shows the difference in the infestation of Cheviots and Blackfaces exposed on the same pastures for the same period. The figures represent the averages for several sheep; they show that when first exposed, the Blackfaces became more heavily infested than the Cheviots, and remained so until May, when the numbers of ticks on both breeds gradually decreased.

Some shepherds believe that the degree of pigmentation of the skin is correlated with the liability to infestation. With a view to testing this opinion a number of sheep, examined for ticks at different times of the year, were classified according to the presence or absence of skin pigmentation, and the counts compared. The evidence obtained did not suggest any correlation.

Geographical distribution.

Nuttall (1911, p. 152; 1916, p. 331) records this tick from Europe (nearly all countries), North Africa (Algeria, Tunisia), North Arabia, Transcaucasia, Kashmir, Japan, North America (Canada and U.S.A.), showing that the species is confined to cool or temperate climates. Senevet and Rossi (1926) lay stress on this; they add some newer locality records.

The following records for Scotland represent information supplied by sheep farmers in the different localities. An asterisk (*) indicates that no specimens were sent in for identification. The signs under "Information" signify: 0 absent, +? very rare, + present, ++ very numerous.

Distribution of Ixodes ricinus *in Scotland.*

Area	District	Records	Information
Caithness	Dunbeath	1	0
	Watten	1	0
Sutherland	Rogart	1	+
	Loth	1	0
	Lochinver	1	+
Ross-shire	*Poolewe	1	+
Elgin	Elgin	1	0
Central Highlands	Pitlochry	1	+
	Aberfeldy	2	+
	"		0 "none in district"
	Rannoch Station	1	+
	Crieff	1	+
	Comrie	1	+
	Auchterarder	1	0
	Stirling	1	0
	*Kilsyth	1	+?
	Balquhidder	2	+
Argyll	Glendaruel	1	++
	Corpach	1	+
	Bridge of Orchy	1	0 "only one or two seen in last ten years"
	Taynuilt	2	+
	Lochgilphead	2	++
	Campbeltown	1	+

Distribution of Ixodes ricinus *in Scotland* (cont.)

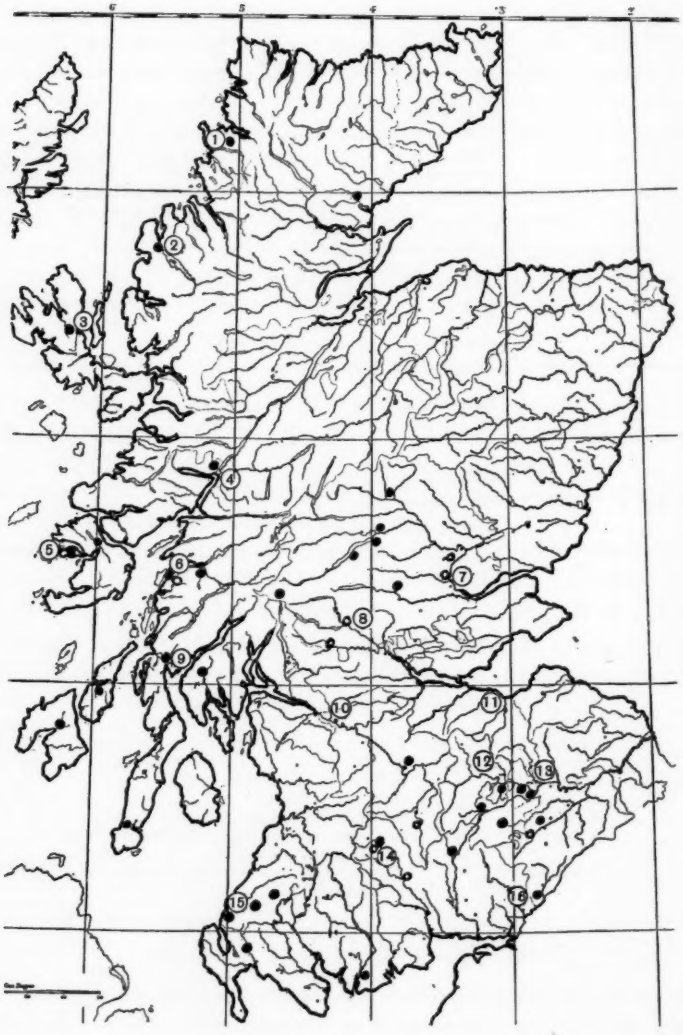
Area	District	Records	Information
Western Isles	Canna	1	0
	Jura	2	+
	Islay	1	+
	Ulva	1	+
	*Aros, Mull	1	+?
	*Portree, Skye	1	+
Central Lowlands	Lanark	1	+ "not very prevalent"
	Innerleithen	2	0 "none north of Tweed"
	Traquair	1	+ south of Tweed 0 north side
	Clovenfords (3)		
	(*a*) South Bank of Tweed	2	+
	(*b*) North Bank of Tweed	1	0
	West Linton	1	0
	Biggar	1	0 "one occasionally hears of isolated cases"
	Cobbinshaw	1	+?
Southwest Scotland	Sanquhar	1	+ "on all farms along Nith"
	*Kirkcudbright	1	+
	New Luce	2	++ "Every hill farm in county infested"
	Lochwinnoch	1	0
Borders	Newcastleton	2	+
	*Moffat	1	+
	Kelso	1	0
	Yetholm	1	+
	Ettrick	8	+
	Hawick	4	+

In addition to the above information for actual localities, the following general information has been supplied, largely by veterinary practitioners.

District	Information
Oldmeldrum	0
Keith	0
Lower Banff	0, in Lower Banffshire
Arbroath	0, very few sheep
Dundee	0, only feeding sheep
Ayrshire	+, parishes of Barr, Ballantrae and Colmonell infested
Berwickshire	0, from Lauderdale along Lammermuirs to the sea

Nature of pasture in relation to I. ricinus.

On an infested farm, the distribution of the tick is governed by the type of soil and ground cover. On comparing the number of nymphs collected from different areas by the blanket method, it was found that ticks were most numerous on pasture consisting of damp ill-drained hollows with soil having a distinctly acid reaction, and covered with rushes and rank vegetation. The tick is also fairly numerous on drier pastures where there is fairly coarse vegetation, with tufts of old *Nardus*, and moss. This is the common type of hill grazing. The soil has a slightly acid reaction. Permanent pasture, the soil of which has a neutral or alkaline reaction, appears to be unsuitable for the tick.



Map. Distribution of *Ixodes ricinus* in Scotland. New records represented by black spots. The open circles are the records of previous workers (as cited by Nuttall, *Monograph on Ticks*, p. 152.)
Places indicated on map by numbers:

Lochinver	1	Isle of Ulva	5	Lochgilphead	9	River Tweed	13
Poolewe	2	Oban	6	Glasgow	10	Sanquhar	14
Portree	3	Perth	7	Edinburgh	11	Ballantrae	15
Fortwilliam	4	Callander	8	Peebles	12	Langholm	16

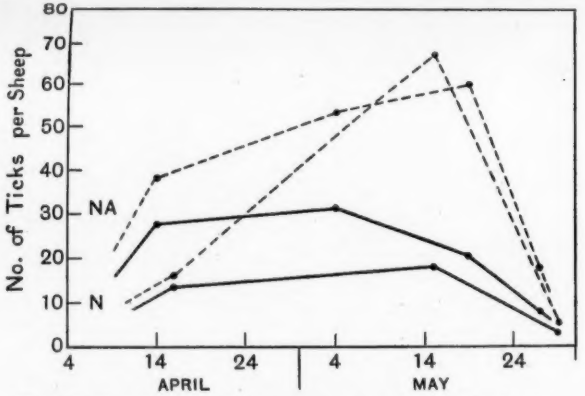


Chart I. Comparative infestations of native sheep (*N*) and non-acclimatised sheep (*NA*).

——— Females } Ticks.
- - - - - Nymphs }

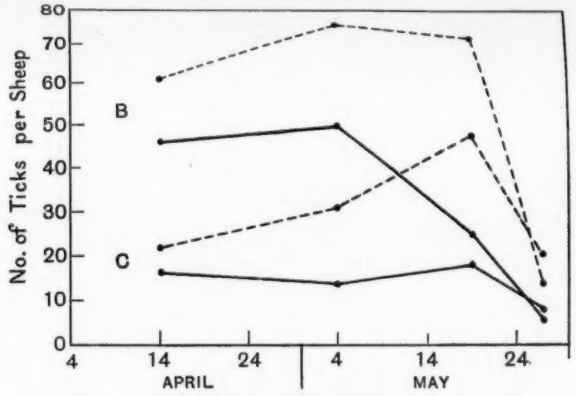


Chart II. Comparative infestations of Cheviot (*C*) and Blackface (*B*) sheep.

——— Females } Ticks.
- - - - - Nymphs }

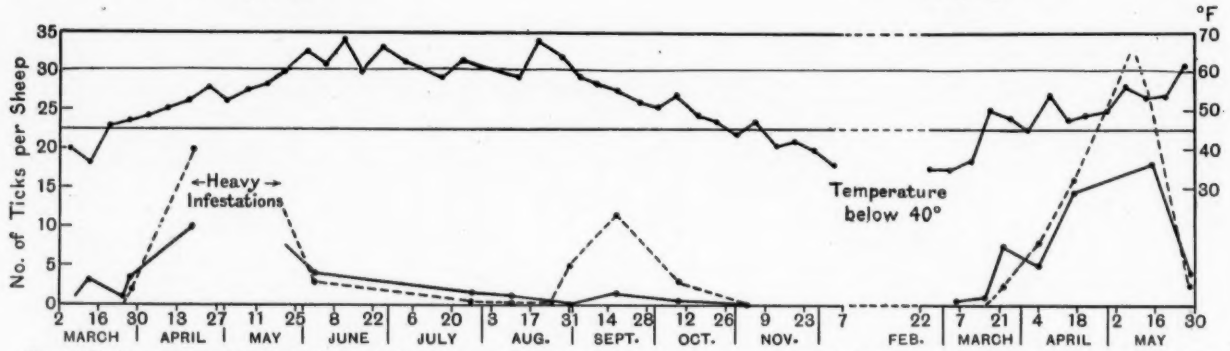


Chart III. Seasonal periodicity in tick activity and temperature variation. The two parallel light lines indicate the limits within which the ticks are active.

——— Weekly average of daily maximum temperature.
——— Females } Ticks.
- - - - - - Nymphs }

Seasonal prevalence on sheep.

Two hill farms, one in the Ettrick valley and one in Taynuilt, Argyll, were visited at fortnightly or monthly intervals during a period of 15 months, and counts were made of the number of ticks found on representative samples of the sheep stock, several sheep being examined in the course of each visit.

The counts, which began in March, showed a sharp increase in number of ticks during April–May. In June the number began to decrease and fell steadily until late August, when there was a slight increase. This autumn rise was of short duration, lasting only until the end of September, after which the number of ticks on the sheep decreased rapidly until by mid-November the sheep were free from ticks. In the middle of March the ticks first began to appear, their number rising sharply through April as in the previous year. By mid-May, the number began to decline, and the ticks were comparatively scarce by June (Chart III). An occasional tick may be found in November and even December, if the weather be mild.

The adults were the first to appear, females attaching themselves to the sheep early in March. Males were found in numbers almost equal to those of the females, and coupled with the latter, or wandering on the wool of the sheep. The nymphs began to appear towards the end of March, and rapidly outnumbered the adults. Their number remained greater until June, when the adults again predominated. In autumn the nymphs were the more numerous. Larvae were found throughout the tick season, but appeared to be most numerous in May–June.

The autumn recrudescence of activity was common to both districts, but was more pronounced in Argyll than in Selkirk. This is of interest, in view of the assertion that there is an autumn outbreak of louping-ill in Argyll, which does not occur, or, at least, is not so marked, in the Border district.

Influence of rainfall and temperature upon tick prevalence.

In 1930, there was a distinct seasonal variation in tick prevalence, but when records of ticks collected from sheep were plotted on a graph in relation to variations of recorded rainfall, no correlation was observed. We may therefore conclude that rainfall is not a factor in controlling tick prevalence on sheep.

Temperature.

Chart III records the air temperature (*weekly averages*) from March, 1930, to May, 1931, and the appearance of ticks on sheep during the same period.

During the first increase in tick activity, from mid-March to mid-May, the temperature was about 40–60° F. From the latter end of May until the first half of August, when ticks showed little activity the temperature was about 60–70° F. From mid-August to mid-October, when there was a second phase of increased activity the temperature ranged between 68 and 50° F., being under 60° F. except for the last week of August. From mid-November until

March the temperature was under 40° F. During the spring of 1931, when ticks were numerous on sheep, the temperature remained between 45 and 60° F., until by the end of May, when the number of ticks declined, the temperature had passed the 60° F. limit.

This seems to show that ticks are more active at 45–60° F. than when 60° F. is exceeded.

That temperature influences tick activity under natural conditions is evident from a study of daily infestations of sheep. Chart IV shows the average daily infestation of nymphs on a number of sheep during April and early May, 1931, plotted against the daily temperature for that period. The figures are only approximate, being based on the daily collection of gorged nymphs from the head and ears of a number of sheep. In the calculation of the

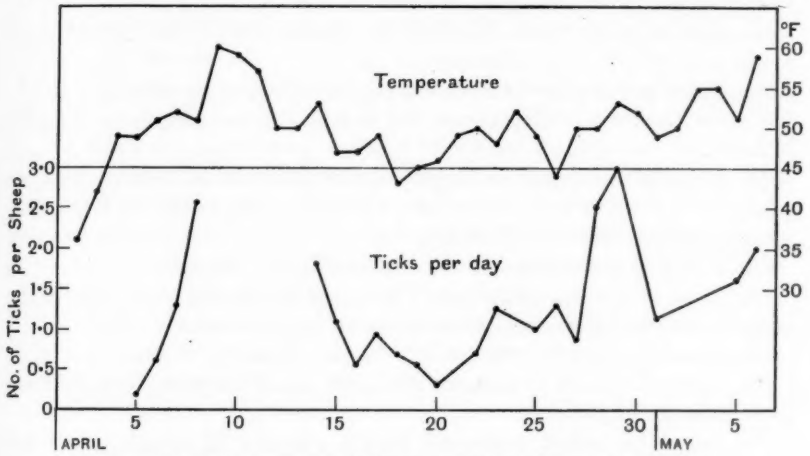


Chart IV. Daily fluctuations in temperature and in degree of infestation.

comparative daily infestation five days have been allowed for the period of engorgement.

It will be seen that when the temperature was under 42° F. there was no infestation, and that the lowest average infestations occurred when it was under 50° F.

It may be tentatively inferred from a study of Charts III and IV that the lower critical temperature for tick activity under natural conditions lies between 40 and 45° F., the optimum temperature between 45 and 60° F. and the higher critical temperature between 60 and 70° F. It cannot be claimed however, that this inference is based on unquestionable evidence, since the influence of other environmental variables on the activity of ticks is not known.

Insect enemies of I. ricinus.

A chalcidid fly *Ixodiphagus caucurtei* du B. was found by Brumpt, parasitising the nymphs of *I. ricinus* in France, and described by du Buysson

(1912). This parasite has since been found parasitising other species of ticks, and attempts have been made to establish the parasite in the United States with a view to the possible control of *Dermacentor andersoni* Stiles. The parasite has not been observed in this country as yet. Over one thousand gorged nymphs, and many gorged larvae and adults of *I. ricinus* collected from sheep in the Ettrick Valley in the spring of 1931, have been bred in the Laboratory, but not one showed evidence of being parasitised by Ixodiphagus or any other insect.

Summary.

Life history of Ixodes ricinus.

1. Under laboratory conditions, at 60–70° F. the pre-oviposition period was usually found to last 15–22 days.

2. Eggs began to hatch after about 4 weeks at 60–70° F. and after 37 weeks at 50–60° F. in the laboratory.

3. Unfed larvae placed on a hedgehog mostly dropped off gorged in 88–120 hours (shortest feeding period 67½ hours). Larvae gorged on sheep in 65 hours.

4. From larvae gorged in August and kept in the laboratory, nymphs emerged after 6–7 weeks. From larvae gorged in September and October, nymphs emerged after 28–37 weeks.

5. Unfed nymphs gorged in 4–7 days, mostly in 5 days.

6. From nymphs gorged on sheep in spring and summer, and kept in the laboratory at 60–70° F., adults emerged after 10–19 weeks.

7. Females gorged on sheep in 7–11 days, mostly in 8–9 days.

8. Sex ratio: about 5: 9 (basis: 362 males and 671 females emerged from nymphs in the laboratory).

9. Longevity unfed: males and females survived 21 months; one male survived for 31 months in the laboratory. Larvae survived 2 years; some were able to feed after fasting 15 months, and nymphs after fasting 13 months.

Bionomics.

10. Hosts: one or more stages of *I. ricinus* have been found on the following hosts. *Mammals:* horse, dog, fox, hedgehog, hare, rabbit, stoat. *Birds:* hoodie-crow, rook, jackdaw, starling, golden plover, barn owl, kestrel hawk, and merlin hawk.

11. When Blackface and Cheviot sheep were kept on the same pastures in spring, the former became more heavily infested with ticks than the latter. It is said by shepherds that ewes weakened by lambing are more liable to infestation than strong barren ewes. Comparative counts of the infestations of a barren ewe and two ewes with lamb appear to confirm the shepherds' view. Non-acclimatised (experimental) sheep, when exposed on a tick infested farm, rapidly lost condition at first, and subsequently began to recover. When these sheep, whose health and general condition were low, were compared with native

sheep their average infestation with ticks was found to be higher than that of the natives. As the health and weight of the experimental sheep improved, the average infestations of these and of the native stock approximated.

12. Reports on the geographical distribution of *I. ricinus* in various districts in Scotland, received from sheep farmers and others, are recorded.

13. There is a distinct seasonal periodicity in the activity of *I. ricinus*. It appeared on sheep early in March, and became increasingly numerous during April-May. In June the numbers began to decrease, and steadily diminished until late August, when there occurred a slight increase in average infestation of sheep. This increase was of short duration and by the middle of November no ticks were found on sheep. The same periodicity was observed in the following spring and summer. An occasional tick was found on sheep during the winter, in mild warm weather. Adult ticks first appeared in early March. Nymphs appeared by the middle of March. Larvae were apparently most numerous in May–June.

14. The seasonal incidence of *I. ricinus* on sheep in 1930 bore no apparent relation to rainfall.

15. When the variations in average weekly air temperature for 1930–31 are charted in relation to the seasonal incidence of ticks on sheep for that period and the curves compared, there seems to be a correlation between temperature and tick activity. The data indicate that the tick infests sheep in the greatest numbers at 45–60° F. The lower critical temperature for tick activity in nature appears to be 40–45° F., and the higher 60–70° F.

16. Over a thousand gorged nymphs and many larvae have been collected in the field and bred out in the laboratory; in none of them has there been any evidence of hyperparasitism.

Acknowledgments.

I wish to express my sincere gratitude to the Director, Dr J. Russell Greig, and to the staff of the Institute, for their encouragement and ready aid in the prosecution of this enquiry.

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I have to record my indebtedness to Prof. G. H. F. Nuttall, for his advice on the handling of ticks, and the preparation of the manuscript, and also for the determinations of specimens.

In conclusion I should like to express my sincere appreciation of the helpful criticism and advice received from Dr A. E. Cameron, and from Dr R. Stewart MacDougall.

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THE DEVELOPMENT OF WEST AFRICAN STRAINS OF *TRYPANOSOMA GAMBIENSE* IN *GLOSSINA TACHINOIDES* UNDER NORMAL LABORATORY CONDITIONS, AND AT RAISED TEMPERATURES.

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(With Plate XVII, containing 4 Figures.)

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I. Introduction.

The difficulty of infecting species of Glossina with *Trypanosoma gambiense* under laboratory conditions is well known. Wenyon (1926) remarks that the percentage of *G. palpalis* in which the complete developmental cycle of *T. gambiense* can take place varies but is well under 10 per cent. Hoare (1931 *a*) puts the normal infection rate at about 3 per cent. Duke (1930) states that "With Glossinae exposed to trypanosomes of the *brucei* group, 10 per cent. of infected flies is a high figure." The same author (1928 *a*) describes work in which nineteen strains of *T. gambiense* were studied and their infectivity to *G. palpalis* estimated. Of these nineteen strains, seven failed to complete the cycle of development in the tsetse and were termed non-transmissible; the remaining twelve strains gave mature infection rates in the fly of from 0·6 to 6·9 per cent., the total infection rates (including immature infections) in the same strains varying from 1·9 per cent. to 10·1 per cent.

This low infectivity of the polymorphic trypanosomes to Glossina is most remarkable and has given rise to some doubts as to the importance of the cyclical method of transmission of *T. gambiense* by Glossina particularly in epidemic areas where sleeping sickness may spread so rapidly as to suggest a

simple mechanical mode of transmission. Duke (1919, 1921, 1923 *a*, 1923 *b*) has put forward arguments in favour of such a view.

The difficulty which *T. gambiense* seems to encounter in establishing itself in Glossina is in striking contrast with the ease with which most non-pathogenic trypanosomes infect their invertebrate hosts. Moreover, such pathogenic trypanosomes as *T. vivax* and *T. congolense* infect a high percentage of experimental tsetse under laboratory conditions. In Northern Nigeria infection rates in *G. tachinoides* average about 60 to 70 per cent. with *T. vivax* and 15 to 35 per cent. with *T. congolense*. Hoare (1931 *a*) suggests that the susceptibility of insect vectors to trypanosome infection is of biological significance as indicative of the phylogeny of the trypanosomes. According to this view the non-pathogenic trypanosomes with a posterior station in the insect host and contaminative method of infection are phylogenetically oldest, while the polymorphic group of the pathogenic trypanosomes with anterior station and the inoculative method of transmission are still very imperfectly adapted to their invertebrate host, and this is evidenced by their low infectivity to the insect vector.

An important fact, mentioned by Robertson (1912) and recently emphasised by Duke, is that strains of *T. gambiense* vary in their power of development in Glossina. This power of cyclical development in Glossina has been designated transmissibility by Duke, and this author has introduced a Transmissibility Index which he believes may be regarded as characteristic for each strain tested. Transmissibility differs from the general infectivity of a strain to tsetse in that it ignores light gut infections in tsetse, these being of the nature of simple cultures in the gut which die out sooner or later without developing further. Transmissibility, according to Duke (1930), is regarded as dependent both on the natural resistance of Glossina to infection and also on the production by the *T. gambiense* strain of adequate numbers of a hypothetical infective form, which form alone is capable of infecting tsetse. It is an unstable character which under certain conditions, such as prolonged direct passage or prolonged sojourn of a strain in a single host, may be reduced or even completely lost. That is to say, the power of completing the developmental cycle in the tsetse with salivary gland and proboscis infection and, finally, even the power of infecting the gut, may disappear. If this total loss of transmissibility occurs in nature with any frequency, and Duke's analysis of East African *T. gambiense* strains suggests this, then it would seem that *T. gambiense* strains are constantly becoming extinct, a conclusion of very considerable importance from the epidemiological standpoint.

Lloyd (1930), in discussing results obtained from *T. brucei* transmissions in Nigeria, criticises Duke's conclusions on the grounds that the influences of factors such as climate, nutrition and the bacterial flora of the gut on the infectibility of Glossina with trypanosomes of the *brucei* group have not yet been adequately determined: and that until this has been done results of transmissibility experiments carried out under laboratory conditions must be accepted with reserve.

The work to be described was planned in order to study the infectivity to *G. tachinoides* of a representative selection of West African strains of *T. gambiense* with the particular object of determining whether non-transmissible strains occur here as frequently as they appear to do in East Africa. Parallel with this it was decided to continue investigation into possible factors influencing the *T. gambiense* infection rate in Glossina, since it seemed not impossible that some factor or factors necessary for normal development of the *brucei* group of trypanosomes in tsetse had been absent in the transmission experiments carried out hitherto in African laboratories.

II. TECHNIQUE AND METHODS.

Throughout the transmission experiments described below only clean laboratory-bred tsetse have been employed. During the dry season, flies obtained from daily collections of wild puparia were used, while during the wet season, when breeding in the bush is reduced to a minimum, it was only possible to use bred flies obtained from a large stock of breeding flies kept in the laboratory. Under these conditions the great majority of the experimental work has to be confined to the dry season months of November to June.

Bred flies used in transmission experiments were kept in cages of the type described by Lloyd (1930). These cages serve their purpose well and, besides occupying little space, have the great advantage of being very easily applied to any of the experimental animals used and particularly to guinea-pigs.

Infected flies were maintained in a moist chamber where the relative humidity is kept at a high level throughout the year, and the diurnal variations in temperature usual in the dry season are greatly reduced. In this way mortality in the experimental tsetse was reduced to a minimum.

Dissections were carried out on or about the twenty-first day after the first of four consecutive days' infecting feeds, though in the case of several rapidly developing strains it was possible to dissect after 13 to 14 days. After dissecting the first few batches of flies infected with a particular strain it was easy to see when the migration of trypanosomes forwards from the gut was taking place, and the usual day for dissection was chosen accordingly.

In dissection the rapid method described by Lloyd and Johnson (1924) was used, with a slight modification enabling the proventriculus to be examined in addition to gut, salivary glands, labrum and hypopharynx.

Method of estimating infection rates.

The Transmissibility Index introduced by Duke for expressing the infectivity of *T. gambiense* strains to tsetse has not been used in these experiments. With the exception of recent work by Duke (1928 *a*–1930) results obtained by previous workers on the infectivity of trypanosomes to their intermediate hosts have been expressed in simple percentages, and it has not been deemed necessary to depart from this method. The chief advantage which

can be claimed for Duke's system seems to be that it enables infections in flies dying during the experiment, often before the developmental cycle has really commenced, to be given their correct importance in the final figures. Now, since it has been shown (Duke, 1928 *b*) that *T. gambiense* infections have no effect on the longevity of the infected tsetse, it would seem that by ignoring flies which die in the course of an experiment and calculating infection rates from those which survive long enough for infections either to develop or die out (usually about 21 days or less), an equally accurate estimate of the infectivity and transmissibility of a strain may be formed.

Practical disadvantages of dissecting all flies which die in the course of an experiment are that, as Lloyd (1930) points out, flies which have been dead for more than a very few hours become dried and difficult to deal with: and, moreover, are often rendered useless by very heavy bacterial infections (Wallace, 1930).

It has been pointed out (Duke, 1928 *a*) that the total infection rate in a batch of "clean" tsetse infected with *T. gambiense* gives little or no information as to the actual transmissibility of the strain. To obtain this information it is necessary to concentrate attention on those infections which are undergoing true cyclical development, and to ignore mere ephemeral gut infections. In most of the work published on this subject infection of the salivary glands is regarded as the only criterion of cyclical development, and these organs alone are examined in order to obtain evidence of mature infection. This method, however, has several disadvantages. It is well known that some strains of *T. gambiense* take much longer to develop than others, and examples have been cited where infection of the salivary glands does not take place till the thirtieth day or after. Individual infections, too, may for various reasons be much retarded. Further, *T. gambiense* infections in salivary glands are often very light, and the opacity of the glands combined with the very sluggish movement of the gland crithidia makes the occasional missing of an infection inevitable. We have had several instances where a batch of tsetse, in which no gland infections were seen, produced infection in the clean animal on which they had been fed. For these reasons it has seemed desirable to include examination of the proventriculus in the routine dissection. Infection of this organ is easily detected, and is usually extremely heavy. It provides a valuable additional means of detecting maturing infections and is never involved in transitory gut infections.

In the tables given below, infections are classified in four groups according to their position in the fly when dissected. The grouping is self-explanatory, but it may be remarked that hypopharynx infections only include those in which metacyclic trypanosomes are seen; infections of this part of the proboscis with proventricular trypanosomes and crithidia in the process of passage to the salivary glands are not included. It has been necessary to give two distinct infection rates. The first, the "Total Infectivity Rate," includes all infections mature and immature, whatever their position or nature. The second, "Cyclical

Developmental Rate," only includes infections in which true development, as opposed to temporary gut culture of the trypanosome is taking place and forward migration to proventriculus, salivary glands and hypopharynx is in progress or complete.

III. THE DEVELOPMENTAL CYCLE OF *T. GAMBIENSE* IN *G. TACHINOIDES*.

Experimental evidence proving *G. tachinoides* to be a vector of sleeping sickness was first obtained by Lloyd and Johnson (1924). This species of tsetse is responsible for a large proportion of human trypanosomiasis in Northern Nigeria in those regions where it replaces the usual riverine species *G. palpalis*. Lloyd and Johnson (1924) give a description of the development of *T. gambiense* in *G. tachinoides*, the cycle corresponding exactly with that described so fully by Robertson (1913) in *G. palpalis*.

In the course of recent transmission work some new observations have been made, and these, together with fresh evidence on controversial points concerning the exogenous cycle, may well be recorded here. The most noteworthy of these fresh observations concerns the position of the developing infection in the gut. Hitherto it has been generally assumed that development takes place in the lumen of the gut, and no note has been made as to the position of the trypanosomes with relation to the peritrophic membrane. This structure whose origin, position and structure have been admirably described by Wigglesworth (1929), has already been shown to play an important part in determining the position of sundry flagellate infections. The writer, in collaboration with Johnson et al. (1928), showed the gut development of *T. congolense* to be extra-peritrophic, and recently Hoare (1931 *a*) has shown that the later stages of development of *T. grayi* in *G. palpalis* are also extra-peritrophic in position. Examination of fresh material and of sections of *T. gambiense*-infected guts prove conclusively that the gut phase of development of this trypanosome, too, is entirely extra-peritrophic. Invasion of the extra-peritrophic space, presumably via the open end of the peritrophic membrane in the hind gut, occurs very early in the infection, and by the fourth day from the infecting meal no trypanosomes are found in the intra-peritrophic space. Once the extra-peritrophic space is infected development proceeds uninterruptedly and the infection normally persists until the death of the fly. The protection which the peritrophic membrane affords to the gut epithelium is obviously shared by the developing flagellates, and it is very probable that their relatively even and uninterrupted course of development may be attributed to their sheltered position (see Fig. 1).

Very many examinations both of the fresh unchanged trypanosome-infected alimentary canal of Glossina and of sections of fixed material at various stages of digestion have convinced us that the peritrophic membrane in Glossina midgut is normally intact throughout the process of digestion, and that the extra-peritrophic development of *T. gambiense* is rarely, if ever, interfered with by

rupture of the membrane. This agrees with the opinions expressed by Hoare (1931 *b*).

Infection of the mid-gut commences at the posterior end and spreads forward until both hind- and mid-sections of the mid-gut are infected. Infection of the posterior region of the anterior section of the mid-gut is seen in heavy infections. From about the tenth day onwards the slender proventricular trypanosomes commence a forward migration, invading the anterior (thoracic) section of the mid-gut and, finally, the proventriculus. Here rapid division and constant invasion produce an extremely heavy infection. Sections of the infected proventriculus from the fifteenth day onwards show in most cases a solid mass of flagellates occupying the cavity of the organ at the point of origin of the mid-gut (see Figs. 2 and 3). Infection extends to the point of origin of the peritrophic membrane between the annular pad and the invaginated fore-gut. Stained smears of the infection in this site have shown that the infection is made up chiefly of long proventricular trypanosomes, filamentar crithidia and rather rare short stumpy crithidia. The proportion of these forms varies according to the age of the infection. Early infections show 100 per cent. trypanosomes while later infections up to the twenty-fifth day show 20 per cent. crithidia. All infections examined, where invasion of salivary glands is in process or complete, show from 5 to 20 per cent. of these filamentar crithidia, which must be regarded as typical of the proventricular infection. These forms measure from 40–50 μ in length and about 0·5 μ in breadth. Robertson (1913) figured these crithidia but regarded them as degenerating forms rarely found in heavy gut infections. Lloyd (1930) has also described and figured them from gut infections of *T. brucei*. In our experience they are usually rare in the mid-gut and may possibly be wandering forms from the proventriculus. The crithidial nature of the proventricular infection is recorded by Brumpt (1927), but Wenyon (1926) follows Robertson in the statement that there is no true crithidial phase outside the salivary glands. Further movement forwards from the proventriculus may take place from the tenth day onwards. No definite observations have been made as to the method by which the flagellates resume their intra-peritrophic position. Flagellates are next seen in the proboscis, and the whole course of the progress from proventriculus to labrum and hypopharynx and thence to salivary glands can be seen in infections of a suitable age. Infection of the labrum appears to be very transitory and has only been noted in cases where the hypopharynx is also infected with proventricular forms. Such infections are rarely seen, and their scarcity would seem to indicate that passage from the proventriculus to salivary glands only takes place at infrequent intervals, probably depending on a combination of favourable conditions.

There seems to be more doubt as to the nature of the forms which invade the glands. Robertson holds that they are trypanosomes of the proventricular type, and Wenyon also adopts this view. Brumpt (1927) states that the invasion is crithidial. To settle this point whole preparations and smears from

infected proboscis have been made in every instance where invasion of the glands was observed to be taking place. The results show that both filamentar crithidia and proventricular trypanosomes are involved, the former type, however, being predominant. In heavy infections of the hypopharynx the lumen may be completely filled by very large numbers of filamentar crithidia, the flickering movement of these extremely long slender flagellates giving a very characteristic appearance to the infection.

The time required for the completion of the developmental cycle, with infection of salivary glands and proboscis, varies, as will be shown later, with the particular strain tested and also with the temperature during development. The proportion of mature infections increases steadily from the time when invasion of the glands first commences. The earliest infection of the salivary glands was seen in a fly examined ten days after the first infecting meal. The time necessary for all infections in a batch of infected tsetse to mature varies considerably; in the rapidly maturing strain Ayu 5, every infection in flies dissected on the twentieth to twenty-first days had reached the glands. In the two strains Ayu 7 and Ayu 8, on the other hand, although the first gland infections were seen as early as the fifteenth day in each case, it was only in batches of flies dissected from the forty-fifth day onwards that 100 per cent. of the infections found (twenty-three in all), were established in the salivary glands, twenty-two of these twenty-three infections showing metacyclic trypanosomes in the hypopharynx.

IV. Comparison of the infectivity to *G. tachinoides* of sundry strains of *T. gambiense*.

In the last section an account is given of the development of *T. gambiense* in *G. tachinoides*. It is necessary to test a number of strains from areas differing as widely as possible climatically and epidemiologically in order to compare their infectivity to tsetse, to determine whether non-transmissible strains occur and, if so, whether commonly: and, also, to see whether any strains show marked divergence from the normal course of development described above. To this end twenty-six strains of *T. gambiense* have been used to infect laboratory-bred *G. tachinoides* and the results of these tests are set forth in Table I. Table II gives the histories of the strains used. The twenty-six strains fall into three groups according to their place of origin. Of these the Gadau series consists of thirteen strains isolated from patients attending the Gadau Sleeping Sickness Clinic and originating from epidemic areas situated in Kano and Bauchi Provinces along the rivers Katagum and Hadejia with their tributaries and the chains of lakes and swamps connected with them. This zone is infested by *G. tachinoides* and limited belts of *G. morsitans* (form *submorsitans*); *G. palpalis* is absent. Human trypanosomiasis here is of a virulent type with a high mortality.

The second group includes four strains isolated from patients in the Gana-

wuri district of Jos Division in Plateau Province. This area, too, is an epidemic one, the vector being *G. palpalis*. An account of tsetse and trypanosomiasis in this area has been published (Taylor, 1930).

The third group comprises nine strains obtained from Ayu District, Southern Division, Plateau Province. This endemic area was especially selected as presenting a strong contrast to the Gadau group. In Ayu the disease is clinically very mild in type and is frequently of very long standing in individual cases. A mass examination of the district carried out in July 1931 by Dr Hope

Table I. *Summary of infection rates in* G. tachinoides *with West African strains of* T. gambiense *under normal laboratory conditions.*

Strain no.	Period of test	Day of dissection	Total flies examined	g.*	g.+pv.	g.+pv. +s.g.	g.+pv. +s.g. +hyp.	Total infectivity rate	Cyclical developmental rate	Result on clean animal
Gadau I	Aug.–Oct. 1929	21	170	—	2	—	1	1·8	0·6	.
,, III	Aug.–Oct. 1929	21	114	—	—	—	1	0·9	0·9	.
,, IV	Jan.–Feb. 1930	20, 21	147	—	1	1	—	1·4	1·4	.
,, V	Feb. 1930	21, 22	181	—	1	1	—	1·1	1·1	.
,, VI	Feb. 1930	21, 22	177	1	—	1	—	1·1	0·6	.
,, VII	Feb.–Mar. 1930	24–26	108	—	—	—	—	—	—	.
,, VIII	Oct.–Nov. 1930	21–23	178	—	1	1	—	1·1	1·1	.
,, IX	Jan.–Feb. 1931	21–22	130	1	3	—	—	3·1	2·3	.
,, X	Jan.–Feb. 1931	20	177	1	7	—	—	4·5	3·9	.
,, XI	Apr.–May 1931	19–20	103	—	—	4	—	3·9	3·9	+
,, XIII	Jan.–May 1931	18, 21	681	3	15	—	2	2·9	2·4	+
,, XIV	Nov.–Dec. 1931	15, 20	283	—	8	1	6	5·4	5·4	+
,, XV	Dec. 1931–Jan. 1932	18, 30	281	1	7	1	—	3·2	2·8	+
Ganawuri I	Nov.–Dec. 1929	26	40	—	—	—	1	2·5	2·5	.
,, III	Jan.–Feb. 1930	20–22	239	1	5	—	—	2·5	2·1	.
,, IV	Jan.–Feb. 1930	21–22	211	—	2	—	—	0·9	0·9	.
,, V	Feb. 1930	22	160	—	6	—	—	3·75	3·75	.
Ayu V	May–Aug. 1931	13–24	143	1	7	3	4	10·5	9·8	+
,, IV	Aug.–Oct. 1931	13–15	152	—	3	2	—	3·2	3·2	+
,, VI	Nov.–Dec. 1931	15–18	213	1	14	1	—	7·5	7·0	+
,, VII	Nov. 1931–Feb. 1932	13–21	388	—	18	6	—	6·2	6·2	+
,, VIII	Nov. 1931–Feb. 1932	15–23	399	—	12	5	—	4·3	4·3	+
,, IX	Dec. 1931–Feb. 1932	11–21	410	1	27	6	—	8·3	8·0	+
,, X	Dec. 1931–Feb. 1932	12–26	378	—	9	4	—	3·4	3·4	+
,, XI	Jan.–Feb. 1932	19–24	332	—	4	1	—	1·5	1·5	+
,, III	Jan. 1932	22	159	—	3	1	1	3·1	3·1	+

*Abbreviations:
g. = trypanosomes in gut only.
g.+pv. = trypanosomes in gut and proventriculus.
g.+pv.+s.g. = trypanosomes in gut, proventriculus and salivary glands.
g.+pv.+s.g.+hyp. = trypanosomes in gut, proventriculus, salivary glands and hypopharynx.

Gill showed an infection rate of 5 per cent. in the total population of 3449. The vector here is *G. palpalis*.

The twenty-six strains, then, originate from both epidemic and endemic areas where the disease is transmitted by *G. tachinoides* or *G. palpalis*.

With the exception of the strain, Gadau VII, when only 108 flies were examined, all the strains were found to be capable of cyclical development in *G. tachinoides*. The infection rates proved to be within the normal range of *T. gambiense* infectivity to tsetse, varying from 0·9 to 11·4 per cent., the corresponding cyclical development rates ranging from 0·6 to 11·0 per cent.

In the Gadau series seven of the thirteen strains were tested in the original

patient, but there was no evidence that this method gives higher infection rates than when the strain is sub-inoculated and tested in monkeys or guinea-pigs. It is essential in these tests to employ large numbers of flies fed on the strain over a reasonably long period in order to strike an average of the infection rates which vary so much at different periods of the endogenous cycle in the vertebrate. This is obviously impracticable where the strain is being tested in man.

A comparison of the strains from the three groups shows that little difference exists in their behaviour in tsetse. Actually the Ayu group of strains proved

Table II. *Histories of strains of the* T. gambiense *used in the present infectivity investigations.*

Strain	Origin: locality and province	*Animal used for test	Length of isolation at time of test in days	No. of animal passages at time of test
Gadau I	Walli, Bauchi	m: 770	41–73	1st
„ III	Hadejia, Kano	m. 805	24–63	1st
„ IV	Kiyawa, Kano	Dodo	—	—
„ V	Katanga, Kano	Isa	—	—
„ VI	Kwarinsa, Kano	Musa	—	—
„ VII	Walli, Bauchi	Gujuri	—	—
„ VIII	Tobon, Kano	p. 24	164–169	3rd
„ IX	Saradugum, Bauchi	Isa	—	—
„ X	Saradugum, Bauchi	Bakura	—	—
„ XI	Bardo, Kano	Ali	—	—
„ XIII	Adabda, Bauchi	(p. B 11, E 5, D 1, T 2, T 3)	258–350	3rd–7th
„ XIV	Gawia, Kano	m. G 14	16–60	1st
„ XV	Makira, Kano	m. 2833	25–49	—
Ganawuri I	Ganawuri, Plateau	Achyat	—	—
„ IV	Ganawuri, Plateau	m. T 4	33–58	1st
„ III	Morwa, Plateau	m. T 1	39–70	1st
„ V	Ganawuri, Plateau	m. T 3	69–71	1st
Ayu V	Ayu, Plateau	m. A 5, m. A 40	29–131	1st–2nd
„ IV	„	m. A 4	135–174	1st
„ VI	„	p. M 21	200–246	2nd
„ VII	„	m. 141	24–69	1st
„ VIII	„	m. 89	27–69	1st
„ IX	„	m. 91	28–69	1st
„ X	„	m. 59	30–69	1st
„ XI	„	m. 60	53–69	1st
„ III	„	m. A 3	274–300	1st

*Abbreviations: m. = monkey.
p. = guinea-pig.

on the average more infectious than the others. It is probable that in seeking the reason why sleeping sickness should be endemic in one area and epidemic in another, one should look for it rather in the nature of contact between the population and tsetse than in differences in the transmissibilities of the *T. gambiense* strains in the two areas.

Since April 1931 the transmissibility of every strain tested has been confirmed by actual infection of guinea-pigs by infected tsetse, and Gadau strains XI–XV and all the Ayu strains have had their transmissibilities confirmed by this additional test.

Although the cycle of development in tsetse was identical in every strain tested, very well marked differences were observed in the time necessary for the completion of the cycle. The most rapidly maturing strains proved to be two from Ayu: Ayu V and Ayu IX.

In Ayu V 25 per cent. of infections in flies dissected on the thirteenth day after the first infecting meal had reached the salivary glands, and metacyclic trypanosomes were seen in the hypopharynx of a fly dissected on the fourteenth day. In Ayu IX 20 per cent. of infections in flies dissected on the eleventh and twelfth days had reached the salivary glands. Dissection was seldom delayed beyond the twenty-first day and by this time most of the strains tested had begun infection of the salivary glands. Gadau XIII, a slow developing strain, showed no salivary gland infections before the nineteenth day and only 18 per cent. of developing infections had reached the glands by the twenty-first day. Gadau XV, tested during the coldest time of the year, did not commence invasion of the salivary glands until after the thirtieth day of infection, and in one batch of flies in which dissections were carried out from the twenty-first day onwards, the first salivary gland infection was seen on the thirty-eighth day. Gadau VIII, IX and X and three of the four Ganawuri strains had not reached the salivary gland stage of the developmental cycle by the twentieth to twenty-third days. It may be significant that these strains were tested at the coldest time of the year when normal development in the tsetse is considerably retarded.

Sufficient early dissections have not been made to make it possible to compare the times of infection of the proventriculus, but such data as have been collected tend to show that infection of this organ usually takes place from the eighth to sixteenth days, depending on the strain and the temperature conditions during the test.

V. Effect of climatic factors on the development of *T. gambiense* in *G. tachinoides*.

A few observations exist as to the effects of climate on the development of pathogenic trypanosomes in *Glossina*. Laveran and Mesnil (1912) in a discussion of the possible influence of climate on the restriction of trypanosomiasis to certain geographical areas cite experimental work by Roubaud, who is quoted as having obtained striking variations in the infection rate of *T. cazalboui* (*T. vivax*) in *G. palpalis* by exposing flies during infection to varying degrees of atmospheric humidity. Similar variations in infection rates were found from examination of tsetse in the dry and wet seasons. Roubaud sums up as follows: "Les trypanosomes sont adaptés à certaines conditions de la salive des mouches, qui varient suivant les influences physiques extérieures; ce sont ces influences qui rendent ou non possible le développement d'un même virus chez un même espèce de *glossine*, et limitent par suite son extension géographique."

Turning now to the *brucei* group, Kinghorn, Yorke and Lloyd (1913) failed

to secure cyclical transmission of *T. rhodesiense* by *G. morsitans* during winter at an altitude of 4000 feet. Fraser and Duke (1912) describe a single inconclusive experiment in which *G. palpalis* infected with *T. gambiense* were incubated at 25° C. to test the effect of this raised temperature on the resulting infection rate. Lloyd (1930) employed a 37° C. incubator in transmissions of *T. brucei* by *G. tachinoides* but obtained no definite evidence that temperature alone affected the infection rate in the tsetse used.

From purely theoretical considerations temperature seems the climatic factor most likely to influence the development of *T. gambiense* in the tsetse. The trypanosome passes the greatest part of its exogenous cycle in the extra-peritrophic space of the mid-gut, in the proventriculus, and in the salivary glands, and in these sites appears to be well protected from the influence of atmospheric humidity—to a greater extent, possibly, than *T. vivax* in the proboscis. On the other hand, fluctuations in the atmospheric temperature are responsible for corresponding fluctuations in the temperature of the body fluids of insects, subject to a very limited control exerted by the living insect itself. By raising the temperature at which the infected tsetse is kept, therefore, one might reasonably expect to be able to affect the behaviour of the trypanosome in the fly. On the theory that *T. gambiense* is still very imperfectly adapted to its invertebrate host, it seemed possible that, by maintaining experimental tsetse at 37° C. during the infecting process, the trypanosome might be better able to establish itself in the fly than when, as usually happens, the trypanosomes taken up by the tsetse experience a sudden drop in temperature from 37° C. to from 20° C.–30° C.

With a view to testing this theory, several strains of *T. gambiense* in *G. tachinoides* have been kept at 37° C. for varying lengths of time during the four days' infecting meals, and the results of these experiments are set forth in Table III. In the experiments conducted during the period May to October 1931, tsetse were kept at 37° C. for 16 hours daily. During the cold season months of November to December 1931, when night temperatures in the laboratory were falling as low as 17° C., this incubation period was found insufficient to affect the infection rates, and the period was increased to 24 hours per day without any serious increase of mortality among the tsetse.

With every strain used, it has been found possible to obtain a greatly increased infection rate by raising the temperature during the infecting feeds. Total infection rates of from 10·5 to 77·8 per cent. have been obtained when the corresponding infection rates in control series kept at room temperature have been from zero to 10·5 per cent., and in several individual batches of incubated flies 100 per cent. infection rates have been recorded.

The mortality in the incubated series of flies has not been excessive and never more than 15 per cent. greater than in the control series.

The practical advantages of a system by which the infectivity of a strain of *T. gambiense* to experimental tsetse may be increased many-fold are obvious. It ensures abundant material being available for studies of the trypanosome in

the fly. It enables several strains of the trypanosome to be maintained in the laboratory by cyclical transmission without having to use very large numbers of bred flies; and it enables cyclical passages generally to be carried out with ease and certainty, even when the transmissibility of the trypanosome under normal laboratory conditions is very low.

The results recorded below are of considerable general interest. They indicate the necessity for estimating the temperature conditions under which tsetse live and become infected with *T. gambiense* in nature: for normal laboratory temperatures probably bear as little relation to those experienced by wild tsetse as do the meteorological records from standard screen readings. Although very little has been done along these lines as yet it may be of interest to note that during the two hot season months of March and April 1931, screen readings taken in the shade of a primary focus of *G. tachinoides* at Gadau showed mean maxima of 43·4° C. for March and 41·1° C. for April. Admittedly

Table III. *Showing the effect on the infection rates of* T. gambiense *in* G. tachinoides *of exposing the tsetse to a temperature of* 37° C. *for* 16–24 *hours per day during* 4 *days' infecting meals.*

Strain no.	Period of test	Flies kept at 37° C. during infecting feeds for	Day of dissection	Total flies examined	g.	g.+pv.	g.+pv. +s.g.	g.+pv. +s.g. +hyp.	Total infectivity rate	Cyclical developmental rate
Gadau XIII	Jan.–Mar. 1931	16 hr. per day	18–21	433	14	57	12	7	20·8	17·5
Ayu V	May–Aug. 1931	16 „	13–19	117	5	52	25	9	77·8	73·5
„ IV	Aug.–Oct. 1931	16 „	13–15	122	2	9	2	1	11·5	9·8
„ VI	Oct.–Dec. 1931	16 „	14–18	248	5	33	12	5	22·2	20·6
„ VII	Dec. 1931–Mar. 1932	24 „	13–50	275	5	27	2	9	15·6	13·8
„ VIII	Dec. 1931–Mar. 1932	24 „	17–50	319	12	32	9	13	20·7	16·9
„ IX	Dec. 1931–Feb. 1932	24 „	11–21	276	5	42	14	2	22·8	21·0
„ X	Dec. 1931–Feb. 1932	24 „	12–26	237	4	18	3	—	10·5	8·9
„ XI	Dec. 1931–Feb. 1932	24 „	19–24	219	9	22	4	—	16·0	11·9

Abbreviations as in Table I.

the high temperatures to which the experimental tsetse were exposed were of longer duration than similar temperatures in nature. The effects, however, are indubitable and further experiments are to be carried out in which exposure to temperatures of varying intensity for periods of varying duration will be made and the optima for tsetse and trypanosome determined.

Table IV shows the temperatures and humidities to which the experimental tsetse were exposed during the year March 1931 to February 1932, and the meteorological records from standard screen readings taken at the Tsetse Investigation, Gadau. The generally lower mean maxima and the higher humidities in the fly cages are noteworthy.

The effect of high temperature during the infecting meals on the subsequent infection rates in tsetse can be regarded as partly due to a simple acceleration of the multiplication by fission of the trypanosomes taken up in the infecting meals, and partly to a reduction in the mortality which always follows the

sudden change of conditions undergone by the blood trypanosome, one of which conditions is the relatively low temperature of the new medium in which it finds itself in the tsetse gut. Dissection of incubated tsetse during the first

Table IV.

Monthly temperatures and humidities in infected tsetse cages, Gadau.

Month	Mean temp.	Mean relative humidity* %	Mean max.	Mean min.	Absolute max.	Absolute min.
1931:						
March	27·0	47	29·5	24·5	32·8	22·2
April	28·7	68	30·0	27·3	31·6	24·5
May	29·2	76	30·0	28·4	31·0	27·3
June	27·4	90	28·8	26·0	31·6	25·0
July	26·2	90	27·3	25·0	28·8	23·9
Aug.	25·8	85	26·5	25·0	27·7	23·9
Sept.	26·2	85	27·3	25·0	28·8	23·3
Oct.	27·2	76	28·4	26·0	28·8	23·9
Nov.	26·2	64	27·3	25·0	28·8	23·3
Dec.	24·7	63	26·0	23·3	27·7	21·7
1932:						
Jan.	22·2	70	23·3	21·0	28·4	18·3
Feb.	23·6	67	25·0	22·2	28·4	18·3

* Calculated from wet and dry bulb readings taken twice daily: 9 a.m., 4 p.m.

Meteorological data: Tsetse Investigation, Gadau.

Month	Mean temp.	Mean relative humidity* %	Mean max.	Mean min.	Absolute max.	Absolute min.	Rainfall (in.)
1931:							
March	28·2	32	39·0	17·3	42·3	12·8	—
April	32·5	33	41·2	23·9	44·5	19·0	0·1
May	32·0	52	39·5	24·5	41·8	21·7	1·07
June	28·6	68	35·0	22·2	38·4	17·3	6·82
July	26·6	75	31·6	21·7	34·5	19·0	11·55
Aug.	26·0	75	31·0	21·0	33·8	19·0	11·52
Sept.	26·0	71	31·6	20·5	34·5	16·5	5·18
Oct.	26·6	48	35·0	18·3	37·8	13·4	0·78
Nov.	24·5	31	35·0	14·0	37·8	8·9	—
Dec.	22·5	28	33·8	11·2	36·7	7·7	—
1932:							
Jan.	19·5	33	28·4	10·5	37·2	6·5	—
Feb.	21·9	25	31·6	12·2	38·3	6·7	—

* Calculated from wet and dry bulb readings in standard screen daily: 9 a.m.

few days following infection shows massive gut infections, very much heavier than those in the control series kept at room temperature.

Prolonged incubation at 37° C. after the concluding infecting meal has the effect of accelerating development of *T. gambiense* in the tsetse but without raising the infection rate further. It seems, therefore, that the difficulty *T. gambiense* has in infecting tsetse is chiefly met with at the very earliest stage

of the infection soon after the blood forms enter the gut. It is then that mortality is highest, and then that a stimulus such as raised temperature has its maximum effect in determining whether the infection develops, dies or feebly persists for a short time.

It is hardly necessary to point out that temperature is only one of several factors, all of which may play more or less important parts in determining infection rates. A most important factor is the type of trypanosome taken up by the fly, this being dependent on the stage of endogenous cycle in the animal from which the tsetse is infected. This factor is responsible for the great fluctuations in infection rates in batches of tsetse fed over a long period on an infected animal, fluctuations which are magnified in the temperature experiments described above.

VI. Discussion.

The investigations described above, apart from furnishing some fresh information as to the developmental cycle of *T. gambiense* in the tsetse, throw a certain amount of new light on the important subject of the transmissibility of this trypanosome by its insect vector. Since in twenty-five out of the twenty-six Nigerian strains tested, true cyclical development in *G. tachinoides* has been found to take place and since there has been no evidence that any of the twenty-six strains are "non-transmissible," Duke's conclusion that such strains occur frequently in nature cannot be confirmed for the West African Sleeping Sickness areas worked in. This is of interest, for the nine Ayu strains tested were isolated in an area where sleeping sickness is of the mildest type and mortality at its minimum. Such an area, comparable with the Eket endemic area described by Scott Macfie (1914) in Southern Nigeria, might be expected to be most productive of non-transmissible strains of *T. gambiense*, since the disease is frequently of long standing in the individuals affected. On the contrary, the nine Ayu strains proved rather more readily transmissible than the average Gadau strain, isolated from an epidemic area.

On the question of "transmissibility," the present work has shown that exposure of tsetse to a temperature of 37° C. for varying lengths of time during infecting meals spread over 4 days has a profound effect on the resulting infection rates. Duke (1930), in a letter in which he criticises experiments in which Lloyd incubated infected flies at 37° C., dismisses this method as "artificial and enervating" and therefore, presumably, as of no significance. Any method, however, by which the *T. gambiense* infection rate in tsetse can be raised from 10 to 75 per cent. (see Table III, p. 412) seems to the writer to be of both theoretical and practical importance. It can hardly be maintained that conditions of climate, nutrition, or space under which experimental tsetse are kept in the laboratory bear more than a very distant relation to those under which the tsetse becomes infected in nature, and it is therefore permissible to experiment with variations of all laboratory conditions in attempts to discover what appears to be the optimum combination for the development of *T.*

gambiense. Until some serious attempt has been made to investigate in detail the climatic conditions experienced by the vectors of *T. gambiense* in nature it is premature to describe any temperature, to which the tsetse can be exposed in the laboratory without a very high mortality, as artificial and enervating. As has been mentioned, the difference between the mortalities in incubated and non-incubated batches of infected tsetse is not great, and in some experiments the incubated actually survived better than the non-incubated. These temperature experiments are preliminary to a more complete investigation.

Atmospheric humidity is a factor which might not be expected to have much influence on the development of protozoa in the liquid media of gut or salivary glands of an insect, and very few observations have been made on this subject in connection with the above experiments. It is a factor which will, however, be taken into consideration in future experiments.

The effect of nutrition on the *T. gambiense* infection rates in tsetse has been the subject of some enquiry. Robertson obtained an infection rate of 15·5 per cent. in *G. palpalis* starved for 12 days after the infecting meal. The proportion of these infections which consisted of ephemeral "culture" infections of the mid-gut as opposed to true developing infections is not stated. Hoare (1931 *a*) obtained similarly raised infection rates with *T. grayi* in *G. palpalis*. While not exposing infected flies to such prolonged periods of starvation, observations have been made here on the effect of variations in the normal feeding system but without any result.

It has been decided to continue the present system by which each infected cage is put on an animal daily during the experimental period.

The trypanocidal action of fresh sera on gut trypanosomes was demonstrated by Johnson *et al.* (1928), and recently Adams (1931) has shown that this action is destroyed very rapidly in the tsetse mid-gut. It is not unlikely that the extra-peritrophic position of developing *T. gambiense* infections in the mid-gut has a definite protective value in delaying contact between the trypanosomes and the constituents of each fresh blood meal.

Hoare (1931 *a*), in a discussion of factors influencing trypanosome infection rates in tsetse, suggests that an active immunity to infection, natural in the case of *T. gambiense* and acquired in that of *T. grayi*, exists in the tsetse fly. While this view is quite tenable, particularly in view of Huff's discovery of a natural immunity in *Culex pipiens* to bird malaria, there is as yet no evidence that geographical races of the same species of tsetse vary in their susceptibility to trypanosomal infection.

Hoare (1931 *a*) concludes that "it is more likely that (the trypanosome's) capacity to develop is the result of the interaction of the properties of the parasite itself and of the conditions obtaining in both vertebrate and invertebrate hosts." This view is supported by the work described above. The "transmissibility" of a strain of *T. gambiense*, regarded as an inherent character of the trypanosome itself, may and does vary with the strain tested, but accurate comparisons of this character could only be made after very thorough

tests at the same stage of the endogenous cycle in the vertebrate (a cycle about which very little is yet known), and under carefully controlled and standardised conditions of temperature, humidity and nutrition. Results from such experiments would enable comparisons of infectivity and transmissibility of *T. gambiense* strains to be made, but even so would yield no definite information as to the actual transmissibilities of the same strains in nature.

VII. Summary.

1. An account is given of the development of strains of *Trypanosoma gambiense* in *Glossina tachinoides*.

Complete development with infection of salivary glands and hypopharynx occupies from 12 to 40 days and upwards, according to the strain of trypanosome used and the temperature conditions during development.

2. It is shown that the first or intestinal phase of development is passed within the extra-peritrophic space and that the lumen or intra-peritrophic space of the gut is not involved.

3. The importance of the proventricular infection as an index of maturing infections is stressed, and it is shown that filamentar crithidia are always present in this organ in the later stages of infection when infection of salivary glands is taking place. It has been proved that these filamentar crithidia may constitute up to 20 per cent. of flagellates in the proventriculus and are partly responsible for the further migration to the salivary glands.

4. An account is given of the infectivity to *G. tachinoides* of twenty-six strains of *T. gambiense* from epidemic and endemic sleeping sickness areas in Northern Nigeria. None of the twenty-six was proved to be non-transmissible and twenty-five of the strains gave Total Infectivity Rates in the tsetse of from 0·9–10·5, the Cyclical Developmental Rates ranging from 0·6–9·8.

5. No evidence has been obtained that strains of *T. gambiense* from chronic cases of human trypanosomiasis in endemic areas are less transmissible by tsetse than strains originating in epidemic areas.

6. An account is given of experiments in which *G. tachinoides* infected with *T. gambiense* were exposed to 37° C. for varying lengths of time. The effect of high temperature during the infecting period is seen in a marked and constant increase in the proportion of tsetse infected. In the case of one strain a total infection rate of 77·8 per cent. was obtained with true cyclical development in 73·5 per cent. of the tsetse used.

7. It is suggested that the temperatures at which experimental tsetse are infected in the laboratory with *T. gambiense* are excessively low, and an investigation is to be made into the optimum climatic conditions for development of the trypanosome in the fly with a parallel attempt to correlate this with the climatic conditions under which wild tsetse become infected in nature.

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EXPLANATION OF PLATE XVII

Fig. 1. Transverse section of mid-gut of *Glossina tachinoides* infected with *Trypanosoma gambiense*, showing a central mass of indigested blood (*b*) bounded by the peritrophic membrane (*p.m.*), with developing trypanosomes (*t*) lying between peritrophic membrane and mid-gut wall. Section made at twenty-first day of infection. Magnification × 110.

Fig. 2. Section of proventriculus of *G. tachinoides* infected with *T. gambiense* showing the extra-peritrophic position of the trypanosome infection at the origin of the mid-gut (*m.g.*) and the masses of proventricular trypanosomes and crithidia (*t*) lying in the proventriculus. Section made at twenty-first day of infection. Magnification × 140.

Fig. 3. Transverse section of the same showing the massive nature of the proventricular infection (*t*). Magnification × 230.

Fig. 4. Longitudinal section of salivary glands of *G. tachinoides* showing unusually heavy infection of *T. gambiense*, gland crithidia and metacyclic trypanosomes (*t*). Section made on the fortieth day of infection. Magnification × 420.

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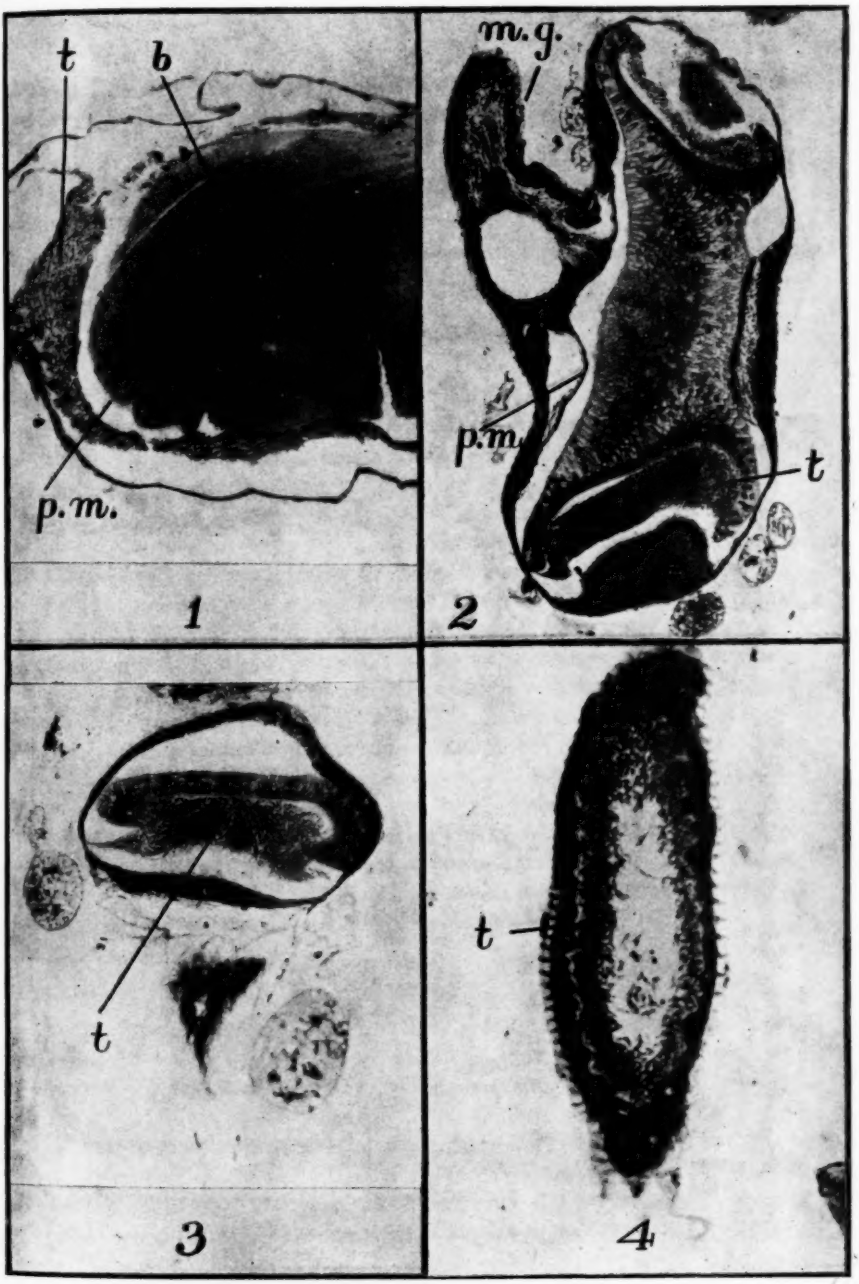
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EFFECTS OF TEMPERATURE AND HUMIDITY ON THE METABOLISM OF THE FASTING BED-BUG (*CIMEX LECTULARIUS*), HEMIPTERA

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(With 3 Figures in the Text.)

The bed-bug, *Cimex lectularius*, inhabits the dwellings of man in temperate regions. It lives in cracks in the walls and furniture, coming out at night to feed; it usually feeds on man, though it will feed on other mammals. The insect gorges itself with blood, only feeding at intervals of several days, and it is able to withstand starvation for periods of several weeks when it is cool.

This paper discusses some of the effects of temperature and humidity on the metabolism of the fasting bed-bug. The rate of loss of water from the insect under various conditions has been studied. The problem is complex, and difficult to approach directly, because the bed-bug not only loses water present in its body at the start of the experiment, but also water made by metabolising food reserves. It is difficult to estimate small quantities of water evaporated into air which is not perfectly dry to begin with. The insects were analysed at the end of the experiments, and from the figures obtained it was possible to estimate how much of the original water had been lost, and how much metabolic water had been produced and evaporated.

The rate of evaporation of water from a number of insects has been shown to be very nearly proportional to the saturation deficiency of the air (see Buxton, 1930, 1931; Mellanby, 1932). An attempt has been made to see whether water is lost by the fasting bed-bug in the same way.

Methods.

The bed-bugs were bred in $3 \times 1\frac{1}{2}$ inch glass tubes, sealed with fine gauze. They were fed once a week on man, when the gauze was held against his arm for about a quarter of an hour. The tubes were kept in moist air in incubators at 23° C.

For experimental purposes, the insects were taken 3 days after feeding, as then they seldom excreted. Except in the preliminary experiments, bugs which had been adult for 1 week only were used, as they were more uniform in body composition, and the females did not lay eggs. To obtain these young

adults, last stage nymphs were sorted out into separate tubes and the adults removed immediately after ecdysis occurred.

The bed-bugs were starved under controlled conditions, and their change in weight noted. These insects only weighed about 4 or 5 mg., and so could not be weighed individually with sufficient accuracy on an ordinary balance. It was not satisfactory to work with batches of bugs, because usually a few individuals died during the experiments. It was therefore necessary to study large numbers of single individuals, and to devise a technique for weighing them accurately. Pieces of fine copper wire were tied round the bugs (Fig. 1), which were then weighed on a torsion balance accurate to $\frac{1}{40}$ mg.—about $\frac{1}{2}$ per cent. of the insects' weight. After the experiment the wire was weighed and its weight subtracted from the gross figures. Bed-bugs appeared to live fairly normally with the wire loops round them—they survived as long as did free individuals, and could feed or lay eggs.

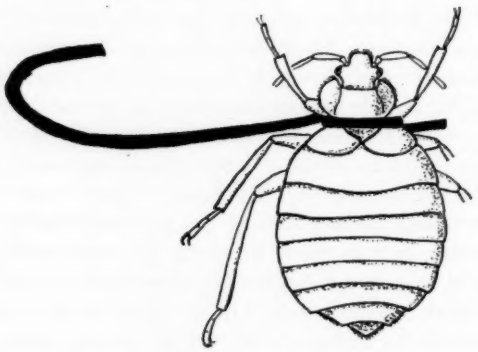

Fig. 1. Bed-bug with wire attached.

The individual bugs with wires round them were placed in separate 3×1 inch glass tubes, which were placed in glass desiccators. The humidities in the desiccators were controlled by mixtures of sulphuric acid and water (Wilson, 1921), and the temperatures by keeping them in incubators.

Loss in weight.

Fasting bed-bugs lived much longer at low than at high temperatures. No accurate data were obtained for the length of life under different conditions of temperature and humidity, but the bugs were kept for periods over which most of a batch survived. This meant that they were kept for different periods at the different temperatures, from 4 weeks at 8° C. to 3 days at 37° C.

On Fig. 2 the rates of loss of weight of bed-bugs kept for 4 weeks at 8° C. and four different humidities are expressed graphically. It will be seen that weight was lost much more rapidly in dry than in moist air. Also, the rate of loss of weight was more rapid during the first week of starvation than sub-

sequently. It might be thought that, after some water had been lost during the first week, the osmotic pressure of the body fluids would be higher and so the remaining water would be held back. But this cannot explain what happened, because the rate of loss of weight decreased least in dry air, where the proportion of dry matter in the insects' body rose to the greatest extent. The rate of metabolism of the insects presumably decreased as starvation proceeded, and this may have reduced the rate of loss of water.

Table I is a summary of the results obtained at five different temperatures. Graphs could be given showing the rates of loss at each temperature, but they would be all similar in essentials to Fig. 2. Further discussion of these results is best postponed until the analyses of the unstarved and starved bed-bugs have been given, when it will be possible to consider the relation between loss of weight and loss of water.

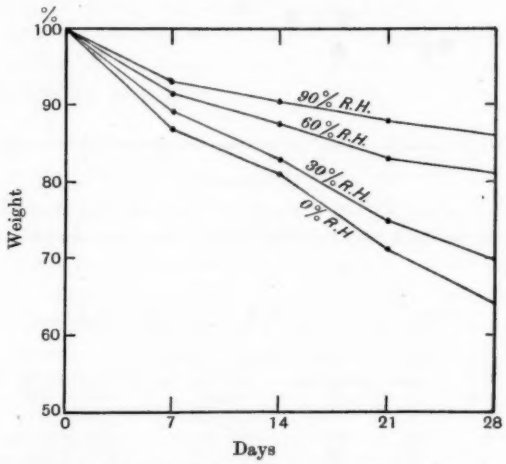

Fig. 2. The loss in weight of bed-bugs at 8° C. and four humidities. Each weight is expressed as a percentage of the weight of that batch 7 days before.

CHEMICAL COMPOSITION OF NORMAL BED-BUGS.

In order to estimate the food-reserves on which the bug lives during starvation, it is necessary to know the normal composition of the insect. Bed-bugs similar to those used on the experiments described above were analysed for dry matter, fat, carbohydrate and uric acid.

(*a*) *Dry matter.*

Individual bugs (with wires round them) were weighed, and then dried at 105° C. to constant weight. After subtracting the weights of the wires from these figures, the proportion of dry matter was calculated. Fig. 3 shows the results obtained with 114 individuals. There is a great deal of scatter, and the pro-

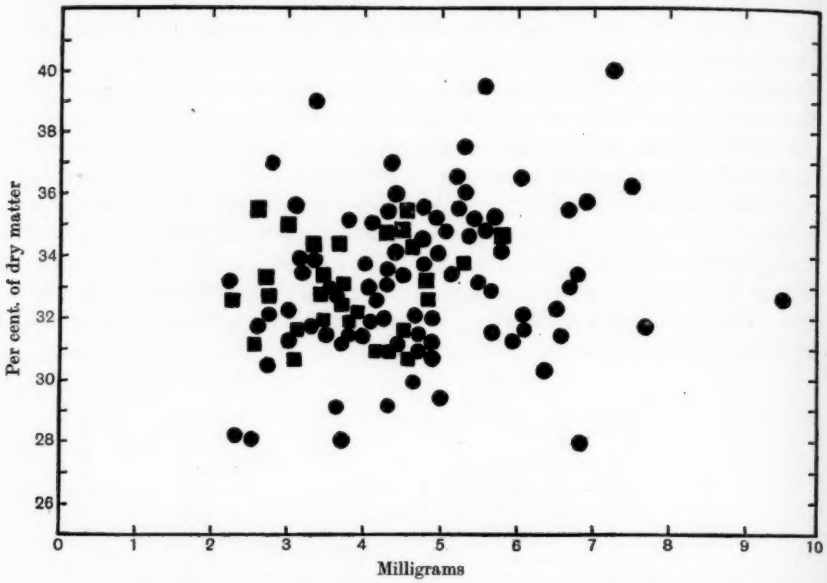

Fig. 3. The proportion of dry matter in 114 bed-bugs. The round dots show the results obtained with insects from the stock culture, and the square those with adults under a week after their last moult.

Table I. *Loss in weight of starved bed-bugs.*

No. of bugs	Days starved	Temperature °C	Relative humidity %	Weight at end as % original weight	Dry matter as % end weight	Dry matter as % original weight
4 (10)	3	37	0	61·0 ± 1·4	51·6 ± 0·7	31·0 ± 0·6
8 (10)	3	37	30	68·3 ± 1·5	43·6 ± 0·8	30·0 ± 0·7
9 (10)	3	37	90	85·0 ± 1·0	36·7 ± 0·42	31·0 ± 0·6
13 (20)	4	29	0	70·6 ± 0·95	39·0 ± 0·61	27·3 ± 0·28
14 (20)	4	29	30	73·0 ± 1·19	37·3 ± 0·67	27·2 ± 0·55
14 (20)	4	29	60	81·6 ± 1·05	34·5 ± 0·42	28·1 ± 0·37
13 (20)	4	29	90	85·2 ± 1·20	33·7 ± 0·67	28·7 ± 0·30
7 (10)	8	22	0	61·6 ± 2·6	45·9 ± 1·4	28·2 ± 1·0
8 (10)	8	22	30	73·9 ± 2·4	38·3 ± 1·3	28·2 ± 0·9
10 (10)	8	22	60	80·7 ± 0·8	37·4 ± 0·6	30·2 ± 0·45
10 (10)	8	22	90	89·0 ± 0·9	32·5 ± 0·6	28·4 ± 0·4
5 (10)	14	16	0	68·2 ± 1·7	41·0 ± 0·91	27·8 ± 0·56
10 (10)	14	16	30	72·2 ± 1·33	40·3 ± 0·67	29·3 ± 0·32
8 (10)	14	16	60	77·0 ± 1·16	38·0 ± 0·63	29·0 ± 0·44
7 (10)	14	16	90	88·0 ± 1·75	32·6 ± 1·04	28·6 ± 0·36
22 (30)	28	8	0	68·7 ± 1·3	45·0 ± 0·67	31·25 ± 0·30
17 (20)	28	8	30	72·5 ± 1·7	43·0 ± 0·95	31·3 ± 0·36
19 (20)	28	8	60	82·2 ± 1·4	35·1 ± 0·85	30·6 ± 0·35
29 (30)	28	8	90	89·1 ± 0·7	33·1 ± 0·36	29·6 ± 0·18

Probable errors are given with all percentages $\left(\text{P.E.} = \frac{\text{S.D.} \times 0{\cdot}674}{\sqrt{N-1}}\right)$.

The figures in brackets in column 1 represent the number of bugs at the start of each experiment. The other figure shows the number which survived, and on which weights, etc., are based.

portion of dry matter appears to be independent of the weight of the individual. Adults taken a week after their last moult, shown by squares on the diagram, have less variation in their composition, and the mean proportion of dry matter is 33·1 per cent. (P.E. 0·18). These young adults ranged from 2·25 to 5·8 mg., and the proportion of dry matter from 30·7 to 35·5 per cent.

(*b*) *Fat.*

The proportion of ether-soluble fat was determined in individual bugs, and also in batches which had been dried and ground up. The individual bugs, on wires, were dried and their dry weight noted. They were then immersed in ether for 24 hours, dried again and weighed. This process was repeated until they reached constant weight—three immersions in ether sufficed. The amount of ether-soluble fat varied widely, reaching nearly 20 per cent. of the dry matter in some individuals, while others had practically none. The difference was not sexual, for both males and females were found containing over 19 and under 2 per cent. of fat. The average amount of fat in 30 individuals was 13 per cent. of their dry matter, and this agreed with the results obtained by extracting batches in the Soxhlet apparatus.

(*c*) *Carbohydrate.*

Preliminary estimations of the carbohydrate present in the bodies of the bed-bugs were made. Dried bugs were hydrolysed with strong hydrochloric acid, and the resulting reducing sugars estimated by v. Issakutz's method. It is realised that the result obtained by this method will be too high, because hydrolysis will give substances which will act like reducing sugars but which do not originate from carbohydrates. But when this preliminary method was tried on starved as well as unstarved bugs, the results obtained indicated that little carbohydrate had been utilised during starvation. Normal bed-bugs yielded an amount of reducing substance equal to about 6 per cent. of their live weight, and the starved ones a proportion of these substances equal to 6 per cent. of their original (unstarved) weight.

These amounts no doubt contain other substances than carbohydrate, but they at least indicate that carbohydrates were not among the principal reserves used.

(*d*) *Uric acid.*

Uric acid is the main excretory product of protein metabolism in insects, and the amount of uric acid produced is a measure of the amount of protein consumed. With the bed-bug, it was necessary to estimate the uric acid in the insects' bodies before and after starvation. They did not usually excrete (three bugs only out of several hundred did so—they were discarded), and so all the uric acid produced must have been retained in their bodies. The uric acid was dissolved out from a batch of dried, ground-up bugs in weak sodium carbonate solution (Wigglesworth, 1925) and estimated colorimetrically by Benedict's method.

Young adults similar to those used in the starvation experiments contained only traces of uric acid (under 0·2 per cent. of their live weight). Older adults were also analysed, and they contained variable quantities, up to 3·5 per cent. being present in some batches.

Chemical composition of starved bed-bugs.

The bed-bugs were starved for different periods at several temperatures and humidities, as described above. After starvation, the proportion of dry matter, fat, carbohydrate and uric acid was estimated in the same manner as with the unstarved bugs.

(*a*) *Dry matter.*

The results are given in the last column in Table I. It will be seen that, at each temperature, substantially the same amount of dry matter has been used up irrespective of humidity. The proportion of dry matter rose considerably in those bugs which were starved in dry air and it appears that the bugs survive longest in moist air, where their ratio of water to dry matter remained nearly constant. The first column of Table I shows that in almost every case more bugs survived the periods of the experiments at 90 per cent. relative humidity than at 0 per cent. though the number of deaths is not sufficient to prove that this is significant.

(*b*) *Fat.*

As the unstarved bed-bugs contained such variable quantities of fat, and some contained practically none, it would have been surprising if fat had proved to be the main food reserve. Analyses of individual starved bed-bugs gave results very similar to those obtained with the unstarved, and batches of dried bugs contained on an average 14 per cent. of ether-soluble fat. So it appears that little if any fat is used during starvation.

(*c*) *Carbohydrate.*

The preliminary analyses of starved bed-bugs for carbohydrate reserves are described along with the analyses of the unstarved, and indicate that reserves of this type were not extensively utilised.

(*d*) *Uric acid.*

The uric acid in the dried carcases of starved bed-bugs was estimated. The bed-bugs starved for 4 weeks at 8° C. were found to contain 2·3 per cent. of uric acid, and they lost approximately the same amount of dry matter. In all cases the amount of uric acid in the starved bugs was practically equal to the loss in solid matter. Now when an insect is using protein, for every 2 grm., burned it produces 1 grm. of uric acid, 1 grm. of water, and some CO_2 (Babcock, 1912). As these insects contained an increase in uric acid equal to the net loss in dry matter, it means that the principal reserve used during starvation was

protein. As the main source of reserve material was presumably the blood which they took at the last meal and stored in the intestine, this result is not unexpected.

PRODUCTION OF METABOLIC WATER.

It has been shown that the chief food reserve used by the fasting bed-bugs was protein. Now as 2 grm. of protein yield 1 grm. of uric acid and 1 grm. of water (in addition to CO_2 which lost in respiration), metabolism itself does not produce any change in weight. Any loss in weight is due to evaporation of water, provided that the bug does not excrete.

This was verified experimentally. A batch of 50 bugs weighing 192 mg. were put in a tube in a stream of dry air at 30° C. The water given off was collected in dry $CaCl_2$. The bugs lost 30·2 mg. in 2 days, and 29·7 mg. of water were collected. Moreover when bugs were kept in air which was completely saturated, their weight did not change, although they were using up solid matter and their ratio of water to dry matter rose.

THE LOSS OF WATER FROM THE BED-BUGS.

A surface of water, in still air, at any temperature, evaporates water at a rate nearly proportional to the saturation deficiency. Certain insects lose water in a similar manner, and it is possible that the bed-bug does so also. If two objects under different conditions are losing water at rates proportional to the saturation deficiency, then the expression $\frac{\text{loss of water}}{\text{saturation deficiency}}$ should be constant; if the period of the experiment is not the same in each case, then $\frac{\text{loss of water}}{\text{saturation deficiency}} \times \frac{1}{\text{time of exposure}}$ should be constant.

It has been shown that the loss of weight of a fasting bed-bug is equal to the weight of water evaporated, so it is possible to work out the expression given above for the bed-bug using the figures given in Table I. The results are shown in Table II. It is apparent that the result is not constant throughout. It varies between 0·15 and 0·38 when the relative humidity of the air is 0, 30 or 60 per cent., and is much higher in the moister air. The results show that the rate of loss of water is not strictly proportional to the saturation deficiency, for it is relatively higher in moist than in dry air.

Table II.

Temperature °C.	Days	Absolute humidity of saturated air mm.	Expression. Relative humidity % 0	30	60	90
37	3	48	0·27	0·315	—	1·04
29	4	30	0·24	0·322	0·38	1·25
22	8	19·8	0·242	0·233	0·302	0·69
16	14	13·6	0·168	0·21	0·306	0·63
8	28	8	0·15	0·179	0·201	0·49

The expression $\frac{\text{loss of water}}{\text{saturation deficiency}} \times \frac{1}{\text{time of exposure}}$ worked out for the results given on Table I.

If the rate of loss of water is not strictly proportional to the saturation deficiency, is it proportional to any other measure of humidity? It is certainly not proportional to the relative humidity or the absolute humidity. If the rate were proportional to the relative humidity of the air, then, at all temperatures and the same relative humidity, the same amount of water would be lost from a bed-bug in 1 day. This is certainly not the case; for instance, the rate of loss at 37° C. and 0 per cent. relative humidity is thirteen times as great as it is at 8° C. and the same humidity. If the rate of loss were proportional to the absolute humidity, then, as at 8° C. the air contains only 7·2 mm. of water vapour when the relative humidity is 90 per cent., the rate of loss should be greater than with air at 29° C. and with a 30 per cent. relative humidity (absolute humidity 9 mm.). Actually the rate is seventeen times greater in the latter case.

In air of 0 and 30 per cent. relative humidity, the proportion of dry matter in the bodies of the bed-bugs rises considerably as starvation proceeds. At 60 per cent. relative humidity, the proportion rises slightly, and at 90 per cent. it remains constant. This means that, in all cases except the last, the insects are losing more water than they are producing by their metabolic processes. Now the rate of loss into air with a 90 per cent. relative humidity is *relatively* much greater than it is into drier air (the expression on Table II is large), but even so, the insect only loses enough water to keep its dry : wet ratio constant. If the rate of loss were strictly in proportion to the saturation deficiency, then in moist air the insects would become "dropsical."

Comparing the rates of loss of water in dry and moist air, it appears that in dry air the insects conserve their water, but as they have so large a surface and so small a volume they cannot do so perfectly. The loss of water is a passive process, and, as might be expected, is nearly proportional to the saturation deficiency of the air (the expression worked out in Table II varies comparatively little for all temperatures and with relative humidities up to 60 per cent.). In moist air, the insects get rid of more water, proportionately to the saturation deficiency, and keep their wet : dry ratio constant. These differences might be caused by having the spiracles closed most of the time in dry air, and open most of the time in moist. No work has yet been published showing this to happen with insects, but Hazelhoff (quoted by Jordan, 1927) showed that the snail *Helix* keeps its pulmonary aperture open in saturated air, and closed most of the time in dry air. If the spiracles were kept open longer when the relative humidity was 60 per cent. than when it was lower, and longer still when it was 90 per cent., it would explain why the expression rises with rising humidity. These insects are certainly very efficient at losing water into moist air. Experiments were made at several temperatures with a relative humidity of 98 per cent. The bugs lost weight almost as rapidly as they did when the relative humidity was 90 per cent., and the ratio of water to dry matter in the insects did not change appreciably. At 29° C. and 98 per cent. relative humidity, for instance, the bugs decreased in weight by 8 per cent. in 4 days. If from this figure the expression used in Table II is worked out, the result is 3·2; this

means that, proportionately to the saturation deficiency, the loss in weight was very rapid indeed. It is only when the air was completely saturated that the bugs were unable to lose water, and it is difficult to obtain complete saturation. Even when the bugs were put in desiccators containing distilled water, slight changes in temperature apparently prevented the air inside remaining fully saturated, and the bugs were able to evaporate a little water; in these circumstances the proportion of water in their bodies did rise slightly. Special precautions, such as covering the tubes containing the bugs with damp muslin, had to be taken to ensure the air was always fully saturated—under these conditions the bugs did not lose weight at all.

The bed-bug is well adapted for withstanding the conditions of its normal habitat. It generally lives in crevices in walls, where the humidity of the air is high. Under these conditions it is an advantage to be able to evaporate surplus water so easily into a moist atmosphere, and the bed-bug will seldom be exposed for long to conditions which will cause desiccation.

A COMPARISON OF THE EFFECTS OF TEMPERATURE AND HUMIDITY ON *CIMEX* AND *RHODNIUS*.

This work on *Cimex* was done in collaboration with Dr P. A. Buxton, who was simultaneously experimenting with the Reduviid bug, *Rhodnius prolixus*. His work is described on pp. 429–439 of this *Journal*. At this stage, discussion of the results obtained with the two insects is premature, but a short comparison between them is of interest. Both insects are blood-sucking Hemiptera, which gorge themselves at intervals of several days, and can withstand starvation for considerable periods. But a consideration of the results shows that the insects behave differently in many ways.

The difference in size between the bed-bug and *Rhodnius* may well influence and cause differences in the way in which they lose water. Bed-bugs usually weight about 5 mg. and *Rhodnius* about 100 mg. This means that the ratio of body surface to weight is very different in the two animals.

The behaviour of the two insects in the experiments was different. The bed-bug lost weight more rapidly in dry than in moist air, but consumed the same amount of dry matter in each case. As a result, there were great differences in the ratios of dry matter to water in the insects' bodies at the ends of the experiments. *Rhodnius* lost practically the same proportion of its gross weight at all humidities, but used up less solid matter in dry than in moist air. Thus in dry air, with both animals, the proportion of dry matter rose during the experiments, but for different reasons—in the bed-bug because so much water was lost, and in *Rhodnius* because in dry air less solid matter was used up. In moist air both insects retained their original proportions of water and solid matter, but while bed-bugs' weight never fell below 85 per cent. when the air had a humidity of 90 per cent., the weight of *Rhodnius* fell to 50 per cent. of what it was at the start of the experiment. *Rhodnius* got rid of much more solid matter than the bed-bug, partly by excretion.

The similarity in mode of life of these two insects does not appear to influence their metabolism to any great extent, and at present it seems that it is best to consider them separately. It is hoped that at some future time, in the light of further knowledge, it will be possible to compare these insects more fully, and if possible to compare their reactions to temperature and humidity with those of a number of other insects.

SUMMARY.

A method is described by which individual bed-bugs, weighing only 5 mg., can be accurately weighed, and their rate of loss of weight measured during starvation.

Fasting bed-bugs were kept for various periods at five temperatures, ranging from 8° C. to 37° C., and at four humidities—0, 30, 60 and 90 per cent. relative humidity—at each temperature. Analysis after the experiments showed that the same amounts of food reserves were used up at each humidity for one temperature, and, as more water was evaporated from those kept in dry air than from those in moist, the proportion of dry matter rose most rapidly in dry air. Protein was the main food reserve used.

Although the rate of loss of water was greatest in dry air, the rate of loss was relatively greater in moist air when the saturation deficiencies are compared. It appears that the insects conserve their water in dry air, but their surface area being so great in comparison with their volume, they cannot prevent all evaporation. This evaporation is at a rate nearly proportional to the saturation deficiency of the air.

In moist air water appears to be evaporated freely. It is suggested that the spiracles are kept closed more in dry air and less in moist, which accounts for the fact that the rate of evaporation is proportionately greatest in moist air.

A comparison is made between the results obtained with *Cimex* and *Rhodnius*.

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THE RELATION OF ADULT *RHODNIUS PROLIXUS* (REDUVIIDAE, RHYNCHOTA) TO ATMOSPHERIC HUMIDITY.

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(With 4 Figures in the Text.)

The work here described is an attempt to analyse the relation between this blood-sucking bug and the humidity of the atmosphere in which it is kept. *Rhodnius prolixus* is capable of taking enormous meals of blood at long intervals, and it lives by digesting these meals very slowly. I wished to find out whether the loss of weight of fasting bugs, or their duration of life, was influenced by being kept in dry or moist air, and if so, what was the optimum humidity. I wished also to discover whether the bugs possessed any mechanism for regulating the proportion of water in their bodies, as their total weight fell during starvation.

The work has been carried out whenever adults of the right age were available, and has already occupied more that 2 years. It is evident that *Rhodnius* is not a very appropriate insect for investigations of this type, because it passes considerable quantities of faeces at irregular intervals during the first 10 days of the experiment, and I do not propose to continue working with it. *Cimex*, which has been investigated by my colleague Mr Mellanby (p. 419) is clearly the more convenient animal. The paper is a record of fact: a full discussion of the results is impossible, because we can at present only compare *Rhodnius* with *Cimex*, and with the meal-worm, the larva of *Tenebrio molitor* (Buxton, 1930 *a*; Mellanby, 1932). But it is not inappropriate to point out that the method here pursued, though laborious, seems capable of giving results of great interest: indeed it is one of the few methods we have of precisely analysing the effect of temperature and humidity upon an insect.

My thanks are due to my statistical colleagues Miss H. Woods and Dr J. O. Irwin, for advice and for help in computing: also to Mr S. Smith who has carried out most of the many thousands of weighings.

Material.

An account of the biology and the method used in rearing the insect has been published elsewhere (Buxton, 1930 *b*). Individuals in the last larval stage were allowed to feed, taking generally about six times their own weight of

blood. They were then isolated in tubes at 23° C. in a damp incubator and examined daily, until they moulted. This occurs about 3 weeks after the last meal. When the cast skin was observed the insect, known to be less than 24 hours old, was used, either as a "normal," or in one of the experiments described below.

If a *Rhodnius* is dissected a few hours after it has become adult one finds the mid-gut loaded with blood, though it has not fed for about 3 weeks. The hind-gut is sometimes empty, at others it contains an appreciable quantity of urates. It is the irregular deposition of this material which partly accounts for the erratic loss of weight during the first 10 days of an experiment. Wigglesworth (1931) has given a very full account of excretion in *Rhodnius*, discussing the successive stages in the excretion of water and of the other constituents of the urine. But the facts which he gives do not apply precisely to my insects, because he took adults of uncertain age, allowed them to feed and then studied the sequence of events.

Twenty freshly emerged individuals (of both sexes) were taken, and the mid-gut and hind-gut with their contents were dissected out and weighed, then dried at 105° C. and again weighed. The mean weight of the insects was 120 mg.: the mid- and hind-gut together had a mean weight of 48 mg. (maximum 70, minimum 30), and the mean dry weight was 20 mg. The mid-guts and hind-guts of the same twenty individuals were also weighed separately: the mean wet weight of the hind-gut was 6 mg. (range 10·5 to 0·5 mg.), and the mean dry weight 2·5 mg. One may therefore say that the hind-gut averaged about an eighth of the combined weights of mid- and hind-gut, which I have quoted above. An important conclusion may be drawn from these figures. The wet weight of the gut is 40 per cent. of that of the insect; from this we deduct 6 per cent. for hind-gut, and a little for the gut wall itself. There remains about 30 per cent. of partly digested concentrated blood: two-fifths of this (40 per cent.) is solid. But in the whole insect, including the gut, the proportion of solid matter is 30 per cent. (p. 431 below). From this it follows that one hundred parts of freshly moulted *Rhodnius* contains:

Blood in gut	30 parts of which	12	are solids
Rest of insect	70 „	18	„

The proportion of solid matter in the "rest of the insect" is therefore 26 per cent., a figure which should be borne in mind. The proportion of solid matter in the actual tissues is lower still, because a considerable part of the insect is skeleton. The skeleton is 11 per cent. of dry weight (3·1 per cent. of the wet weight) of this insect (Buxton, 1932).

It is clear that the proportion of blood in the insects at the time of the last moult is very great, and as the fat body is small we may conclude that they resist starvation by the slow metabolism of the contents of the alimentary canal. That this is also so in *Cimex* is shown fully by Mellanby (p. 424).

If individuals are isolated from the time of the last moult, they pass faeces

for about 10 days: some do so almost daily, others only once or twice. The wet weight of faeces is not known, but the total dry weight, in eighteen individuals studied, ranged from 13 to 3 mg. (mean 7 mg.). The loss, and the rate of loss, due to defaecation is therefore highly erratic.

NORMALS.

The normal proportion of water in the freshly emerged *Rhodnius* was established by taking individuals known to be less than 24 hours old. They were weighed to 0·5 mg. and then dried to constant weight at 105° C.: for our purposes it is most convenient to express the dry weight as a percentage of the original weight, because we propose later to study the rate of loss of weight. It is assumed that what is driven off is water, though an error is doubtless involved in this assumption (Vinogradov, 1929): the technique has the merit of being standardised and simple. Individuals were killed and treated in the same way from time to time during the course of the experiments: they were not selected. The following facts were established, means and standard deviations being followed in each case by their probable errors.

	Males	Females
Number	48	95
Wet weight, mean (mg.)	110·56 ±1·56	126·55 ±1·36
S.D.	16·03 ±1·10	19·62 ±0·96
Wet weight, max. (mg.)	144	184
min. (mg.)	75	74
Per cent. of dry matter, mean	29·77 ±0·32	30·50 ±0·22
S.D.	3·29 ±0·23	3·14 ±0·15
Dry matter (%), max.	35·5	36·0
min.	19·0*	23·0

* The next lowest percentage being 24.

From these facts (and from Figs. 1 and 2) it is apparent that in both sexes the proportion of dry matter is about 30 per cent. of the wet weight, though one should not forget that in the "rest of the insect," after the partly digested blood is removed, the proportion of solids is about 26 per cent. The degree of individual variation from 30 per cent. is great, and the dry matter ranges from about 24 to about 36 per cent. of the weight of individual bugs. This inconsistency between individuals is also shown by the correlation coefficient (r) between wet weight and proportion of dry matter, which is $0{\cdot}471 \pm 0{\cdot}076$ for the above males and $0{\cdot}558 \pm 0{\cdot}048$ for the females. The correlation, therefore, though significant, is not very high.

The proportion of solids to the wet weight of the insect rises with rising weight. This is easily seen in Fig. 1 or 2 from the position of the individual points on the graph. In the males, for instance (Fig. 1), there is a clear tendency for the proportion of dry matter to be above 30 per cent. in those weighing more than 110 mg., and less than 30 per cent. in those whose weight is below that figure. The lines which cross the face of the graphs show the average percentage of dry matter for given wet weight. For statistical purposes

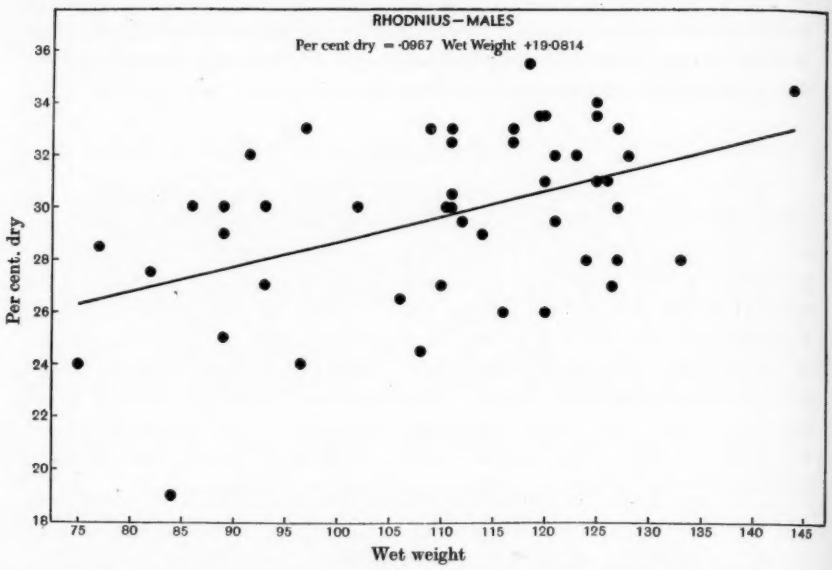

Fig. 1. Relation between weight and proportion of dry matter in 48 male *Rhodnius prolixus* killed when less than 24 hours old. The "regression line" shown in the figure is the straight line which gives the best average "per cent. dry" for a given "wet weight."

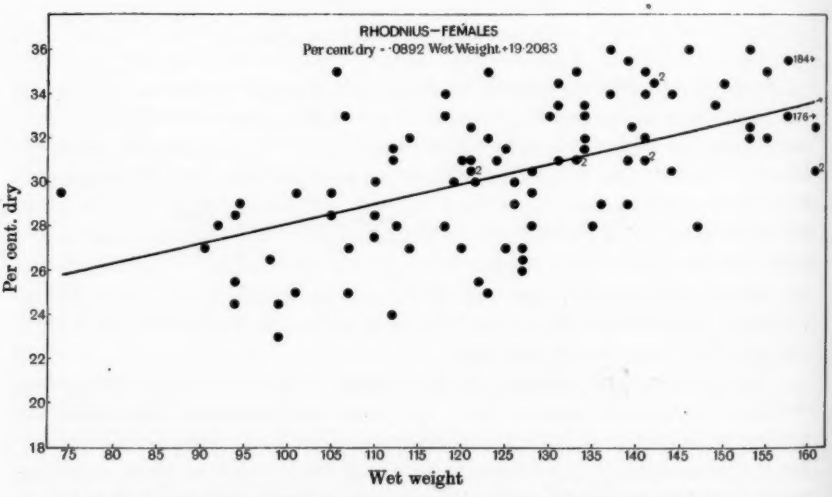

Fig. 2. Relation between weight and proportion of dry matter in 95 female *Rhodnius prolixus* killed when less than 24 hours old. The "regression line" is the straight line which gives the best estimate of average "per cent. dry" for given "wet weight."

this relation is best shown by calculating a regression coefficient which is as follows:

In males, the per cent. dry matter = 0·0967 (wet weight) + 19·0814.
In females, the per cent. dry matter = 0·0892 (wet weight) + 19·2083.

In other words, for each 10 mg. increase in live weight, the average male contains 1·0 per cent. more dry matter, the figure for the average female being 0·9 per cent. One may perhaps assume that this is due to the proportion of skeletal tissues being greater in the larger individuals.

As there is so much difference between individual males, both in weight and in proportion of water, it may be asked whether *Rhodnius* is appropriate for the type of experiment which we contemplate. The difficulty may be partly overcome by weighing individuals, not groups, and so obtaining a statistical measure of the reliability of the means. But there is a further possibility that the ratio of surface to volume may be so much less in the larger bugs than the smaller as to affect the rate of loss of water. It is possible to show that this source of error is slight. The figures for a dozen bugs, all of which have been exposed to the same conditions for the same period, are arranged in order of original weight; one can find no evidence that the smaller bugs have lost weight more rapidly than the larger. The same thing has been tested statistically by correlating the original weight of the insects with the final weight per cent.: this has been carried out on seven sets of insects, which are the same as those referred to in Table I. The correlation coefficients (r) and their probable errors are as follows:

	Relative humidity			
Period	90 %	60 %	30 %	0 %
21 days	−0·378 ± 0·165	−0·186 ± 0·188	+0·561 ± 0·112	+0·386 ± 0·166
42 days	−0·415 ± 0·161	+0·610 ± 0·128	−0·391 ± 0·162	—

It will be seen that some coefficients are positive, others negative; few of them are statistically significant. The conclusion may be drawn that the effect of size (among a population of adult males of the same age) upon the rate of loss of weight is very slight.

EXPERIMENTS.

The experiments were all carried out with males known to be less than 24 hours old, which were weighed and kept in numbered tubes at 23° C. in desiccators. The desiccators contained mixtures of sulphuric acid and water to control the humidity: the principal experiments were carried out at 90, 60, 30 and 0 per cent. humidity. For purposes of experiment, bugs weighing between 80 and 130 mg. were selected and weighed individually two or three times a week; 24 to 30 insects were used in each experiment. The weight of an individual during the first few days falls most irregularly, owing to passing faeces (See p. 430). On the 21st day of the experiment, all the bugs were weighed and about half of them were killed and the dry weight determined. No

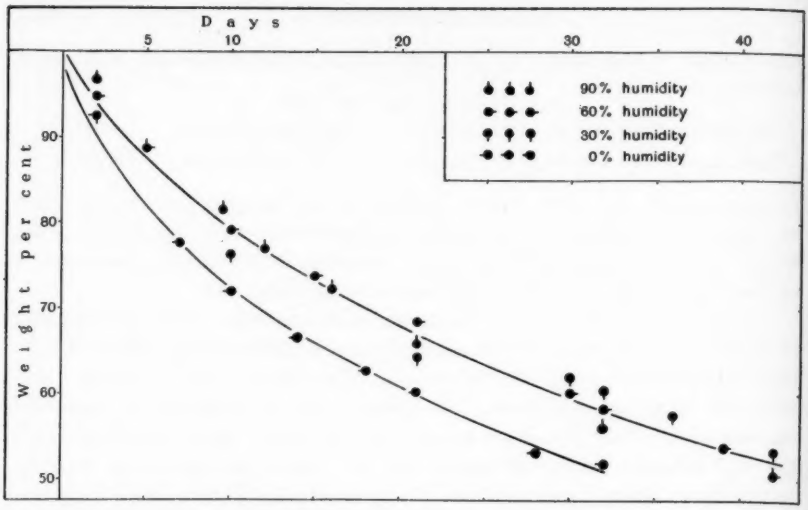

Fig. 3. Mean weights, expressed as percentages of the original weight, of batches of male *Rhodnius prolixus* starved from the last moult at 23° C. and several different humidities. The humidities are distinguished by symbols. Freehand lines have been drawn, the lower through the points shown for 0 %, the upper through the points for the three higher humidities together.

Table I. *Males of* Rhodnius *are starved at 23° C. for certain periods, at controlled relative humidity. Table gives mean weights, expressed as percentage of original weight, after various periods of time: also proportion of dry material in those killed, this being expressed both as a percentage of the original weight, and also of the final weight of the insect at the time it was killed. Means are followed by probable errors, and maxima and minima.*

Day		Relative humidity 95 %	90 %	60 %	30 %	10 %	0 %
21	Number	9	24	23	29	6	30
	Live weight % original	60·41 ± 1·11	65·56 ± 0·69	68·27 ± 0·54	64·66 ± 0·52	61·83 ± 1·0	60·13 ± 0·58
	Max.-min.	68·8 −54·1	75·6 −56·5	73·7 −58·6	71·7 −56·6	66·0 −57·5	69·6 −47·6
21	Number killed	9	12	12	17	6	12
	Dry as % original	18·69 ± 0·39	21·10 ± 0·27	21·43 ± 0·52	20·06 ± 0·36	22·78 ± 0·51	22·61 ± 0·48
	Max.-min.	20·5 −16·3	24·8 −19·3	26·5 −18·0	24·0 −15·8	25·2 −21·0	25·6 −17·8
	Dry as % final	31·04 ± 0·56	32·82 ± 0·27	30·82 ± 0·53	32·24 ± 0·41	37·33 ± 0·44	39·65 ± 0·58
	Max.-min.	34·7 −27·0	35·1 −30·1	36·1 −26·9	36·4 −27·6	38·5 −36·0	44·6 −36·8
32	Number killed	—	12*	11*	12*	—	9
	Live weight % original	—	56·52 ± 0·62	58·00 ± 0·85	60·40 ± 0·57	—	51·77 ± 1·02
	Max.-min.	—	63·0 −51·9	64·5 −51·8	64·6 −52·8	—	58·3 −41·4
	Dry as % original	—	—	—	—	—	21·18 ± 0·49
	Max.-min.	—	—	—	—	—	24·8 −17·2
	Dry as % final	—	—	—	—	—	40·97 ± 0·59
	Max.-min.	—	—	—	—	—	46·2 ±37·3
42	Number killed	—	12	11	12	—	—
	Live weight %	—	50·90 ± 0·73	50·77 ± 1·00	53·17 ± 0·72	—	—
	Max.-min.	—	58·9 −47·0	59·0 −44·0	57·5 −44·4	—	—
	Dry as % original	—	15·33 ± 0·22	17·45 ± 0·33	20·58 ± 0·39	—	—
	Max.-min.	—	17·2 −14·0	20·4 −14·9	24·2 −17·3	—	—
	Dry as % final	—	30·31 ± 0·62	34·28 ± 0·46	38·81 ± 0·80	—	—
	Max.-min.	—	36·1 −25·7	37·5 −31·0	48·3 −33·9	—	—

* These groups were not killed on day 32.

attempt was made to dissect out the guts, and the weights are gross. The remainder were kept alive under the same conditions as before, and weighed from time to time. On the 42nd day they also were killed and the gross dry weight determined. An exception had to be made in the case of bugs kept at 0 per cent. humidity (over P_2O_5), because it was observed that deaths frequently occurred after the 30th day; it was decided to kill this batch of insects on the 32nd and not the 42nd day. Subsidiary experiments at 95 and 10 per cent. humidity were carried out, for 21 days only.

The results obtained in this way are shown in Fig. 3, which gives the mean weights of insects, expressed as a percentage of the weight on the day on which they became adult. The weights are given on a percentage basis, partly because the weights of individual insects differ so greatly, and partly in order to show rate of loss. The means for those starved at 90, 60, 30 and 0 per cent. humidity are distinguished by different symbols. It can be seen at a glance that there is little if any difference between the loss of weight at 90, 60 or 30 per cent. humidity. The loss in dry air (P_2O_5) is more rapid throughout the experiment.

The same results are given, in a more critical manner, in Table I. In this table, each mean is followed by its probable error, calculated as $\frac{\text{S.D.} \times 0{\cdot}674}{\sqrt{N-1}}$, as the value of N is always small. The table gives the live weight after 21 days as a percentage of the original weight: all the means lie between 60 and 68 per cent.: there is no tendency for them to rise or fall as one passes from one end to the other of the humidity scale, and they appear to vary in an erratic manner. In several cases the differences between successive means (*e.g.* between 60·41 at 95 per cent. humidity and 65·56 at 90 per cent. humidity) are significant, but I attribute them to irregularity in defaecation and propose to disregard them. The table also gives the mean weights per cent. after 32, and also after 42 days' starvation. If the mean weights for 32 days are examined, it is found that the differences between those obtained at 90, 60 and 30 per cent. humidity are not significant. But the mean in dry air is significantly different from the others. Taking the available figures for 42 days, the three means lie between 50·90 and 53·17 per cent. and the differences are not significant: no facts at 0 per cent. humidity are available.

At first sight one might conclude that between 90 and 30 per cent. humidity the insect is proof against loss of water, and that the loss of weight is due solely to other causes. But though the loss of weight, of bugs starved at 90, 60 and 30 per cent. humidity, is not affected by humidity, it is possible that the insects at low humidities lose more water and use up less solid matter than those at the higher humidities. That this is the case can be seen from the figures of dry weight of those bugs killed after 21 days, but it is more clear from those killed after 42 days (Fig. 4). The final dry weight of these insects has been taken and expressed as a proportion of their original wet weight: this gives an indication of the loss of solid material during starvation. It will be remembered that the normal bugs contained 30 per cent. of solid matter. After 42 days at

90 per cent. humidity, the proportion is 15·33 per cent. of the original wet weight; at 60 per cent. humidity, 17·45 per cent.; at 30 per cent. humidity, 20·58 per cent. The difference between each of these means and the next one is significant: one concludes therefore that during 42 days' starvation more solid material is oxidised at 90 per cent. humidity than at 60 per cent., and more at 60 per cent. than at 30 per cent.

The same thing can be shown in another way. The normal bug consists of about thirty parts of solid material and seventy parts of water. After starvation for 21 days at any humidity between 95 and 30 per cent., little if any disturbance in this proportion can be observed; the dry weight was between

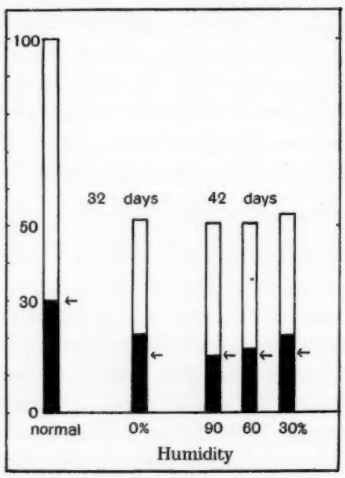

Fig. 4. Proportion of dry matter and water in "normal" male *Rhodnius*, and in batches starved at 23° C. and certain humidities for specified periods. Black is solid matter, and white is water: the arrows show the proportion of dry matter (30 %) which would occur in a normal insect.

30·82 and 32·82 per cent. of the live weight at the time the bug was killed. The figures are not significantly different from one another, or from the normal figure. But those bugs which were killed after 21 days at 10 per cent. humidity contained 37·33 per cent. of dry matter, and those which had been at 0 per cent. humidity contained 39·65 per cent.: the differences between these figures and the normal (30 per cent.) are in both cases significant. The same tendency for the proportion of solids to rise in the bugs which had been starved in dry air is observable in the figures derived from those which were killed after 42 days. But in these experiments, the increase in the proportion of solids is evident, not only at very low humidities but in the bugs which had been starved at 60 and at 30 per cent.: in these the proportion of dry matter was found to be 34·28 and 38·81 per cent. of the weight, and the difference between either of these figures and the normal is significant.

DISCUSSION.

The value of studies such as this will increase when we can compare a dozen or more of different insects. At the present moment comparable data exist only for *Cimex* (Mellanby, p. 419), and the larva of *Tenebrio* (Buxton, 1930 *a*; Mellanby, 1932).

The loss of weight of *Rhodnius* during starvation is not affected by atmospheric humidity (90, 60 and 30 per cent., at 23° C.) even during a fast of 42 days. This must surely be attributed to coincidence, for one cannot suppose that the insect can gain by regulating the rate of loss of gross weight at a time when the proportion of water in its body is undergoing alteration. It is shown that bugs which are kept in drier atmospheres lose more water and also get rid of less solids, so that in two ways the proportion of solid matter in their body rises.

It is possible, though we have no direct proof of it, that the effect of dry air is to keep the spiracles closed, and that this reduces the loss of water, though it still remains greater than it is in moist air: this closing of the spiracles also reduces metabolism, so that the solids are less used in dry than in moist air. We may further suppose that once the increased proportion of solids has been established, in insects kept in dry air, it may be still further increased by viscosity: for as soon as the proportion of solids in the tissues is above normal, viscosity will be greater, so that the rate of diffusion (and perhaps metabolism) will be decreased. But it is clear that viscosity can only increase a difference which already exists, and cannot be regarded as an original cause. A further possibility, suggested by my colleague Dr V. B. Wigglesworth, should be considered. It is clear that *Rhodnius* possesses considerable powers of regulating the proportion of water in its body: these break down after 21 days at 0 or 30 per cent. humidity, and after 42 days at 60 per cent., and any lower humidity. We may suppose that under these circumstances, the body is injured by excessive loss of water, and metabolism falls off because of the injury.

It is also clear (Table I) that *Rhodnius* can tolerate a very great loss of weight without dying. In the experiments in which the bugs were starved for 21 days and in which the weight fell to about 60 per cent. of what it had been, no deaths occurred. In the three experiments in which bugs were starved for 42 days and in which the weight fell to just over 50 per cent. of what it had been, there was only one death and this was apparently attributable to accident. Even in those bugs which were starved over phosphorus pentoxide for 32 days, the number of deaths was only two out of eleven: but when an attempt was made to prolong this experiment to 42 days nearly all the insects died. In subsidiary experiments males under 24 hours old were starved till death at 23° C. At 30 per cent. humidity the mean duration of life of twelve males was $48{\cdot}75 \pm 1{\cdot}51$ days, the range being 36 to 60 days. At 90 per cent. humidity the mean for nine males was $94{\cdot}22 \pm 5{\cdot}02$ days, and the range 70 to 126 days.

Inasmuch as humidity affects the proportion of water in the insect much

more than the gross weight (Table I), we may say that death is caused by increase in concentration of body fluids, and that it appears to occur when the proportion of solids has risen from the normal 30 per cent. to about 40 per cent. But the "normal" figure of 30 per cent. solids is itself deceptive, for it is based on the loss of weight at 105° C. of the whole insect, including the contents of the gut. It has already been shown (p. 430) that if the relatively concentrated gut contents are excluded, the proportion of solids is about 26 per cent. of the insect. The change in concentration which the insect will tolerate is therefore from about 26 per cent. to about 40 per cent. of solids.

This discussion of the facts is very crude. We are not yet in a position to measure the osmotic forces, and the changes in them, which are involved. Nor do we know which of the organs or tissues are most affected by starvation, though it is easy to show by dissection that the alimentary canal is empty and that the volume of the insects' blood is very greatly reduced. It can also be shown that the ovaries and testes remain capable of activity; bugs were starved 34 days, the weight being then about 57 per cent. of what it had been. They were then fed several times, and they paired and laid fertile eggs.

Summary.

1. The paper describes an experimental study on the effect of atmospheric humidity upon *Rhodnius*. The work was carried out on fasting males of known age, kept at 23° C. at different relative humidities; the proportion of water was determined after the lapse of definite periods of time. The normal male contains 30 per cent. of dry matter.

2. Taking the results after 42 days' starvation, at 30, 60 and 90 per cent. humidity, the weight of each group of bugs has fallen to 50 per cent. of what it was at the start of the experiment. Those kept in damp air have lost dry matter and water in the same proportion, so that the percentage of dry matter after starvation is still 30 per cent. of the total weight. Those kept in drier air lose more water but oxidise less dry material, so that the proportion of solids rises during starvation: in those kept at 30 per cent. relative humidity the proportion of solids rises to 39 per cent. The figures for insects kept at 0 per cent. humidity are consistent, but it is not possible to obtain them beyond the 32nd day.

3. Death is probably due to a rise in the concentration of the body fluid and not to the exhaustion of food material. This is shown by the fact that the exhaustion of solid matter is actually less in dry air than in moist, though mortality is only observed among the bugs kept in dry air.

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OBSERVATIONS ON SOME PARASITES OF *OSCINELLA FRIT* LINN.
PART II.

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(With 2 Figures in the Text.)

I. Parasitisation of *Oscinella frit* during 1929 and 1930.

It has already been shown (Imms, 1930) that the stem generation of the frit-fly in the Harpenden district is subjected to natural destruction, to a marked degree, by the agency of certain hymenopterous parasites. Among these, two species, viz. the Cynipid *Rhoptromeris eucera* Htg. and the Chalcid *Halticoptera fuscicornis* (Walk.) were found to predominate in numbers. Parasitisation, to a lower degree, by the Proctotrypid *Loxotropa tritoma* Thoms. was also noted and the Chalcid *Callitula bicolor* Spin. was recorded for the first time as having been reared from this same host. Evidence was also brought forward which appeared to indicate that the parasitisation of frit-fly by the above combination of species increases in intensity as the season advances. In other words the stem generation of frit-fly, attacking late sown oats, is subjected to markedly heavier parasitisation than when it attacks oat sown earlier in the same season.

During the years 1929 and 1930 observations were continued and extended, at Harpenden, along lines similar to those already described, with the object of testing the conclusions previously arrived at. The results to be described in these pages confirm the previous years' conclusions, and also show that there is considerable fluctuation in the relative status, or frequency of occurrence, among the several parasite species present.

In 1929 oats of the variety "Supreme," supplied by Messrs Gartons of Warrington, were sown on a plot 1/8 of an acre in area, which was set apart for the purpose on the Rothamsted farm. The date of sowing was February 28th and the seed was distributed in parallel drills at the rate of three bushels to the acre. In due course samples of affected plants were examined at intervals and, as soon as pupae of the frit-fly were present in larger numbers than the larvae, all plants that appeared to be attacked were removed to the laboratory. The examination and breeding of host material were carried out in a manner similar to that previously described.

During 1930 three plots, each with an area of about 1/10 of an acre, were sown with oats of the variety already mentioned. These plots were adjacent to

one another and formed part of some land, the rest of which itself was under oats. The sowing dates for the three plots were: Plot I, February 29th; Plot II, March 20th, and Plot III, March 29th. For the purposes of collecting material of the frit-fly host, infested plants were removed from these plots and transferred to the laboratory during the following periods: Plot I, from June 19th to 25th; Plot II, from June 27th to July 3rd, and Plot III, from July 7th to 10th.

Records were kept of the dates of emergence of all individuals of both host and parasites, the rearing of these being carried out in the laboratory.

During 1929, 1225 examples of frit-fly were bred and 447 parasites, the percentage of parasitism being 26·7.

During 1930, when three plots were used, the breeding results were as follows:

Plot I: 603 flies, 217 parasites; parasitism 26·4 per cent.

Plot II: 754 flies, 472 parasites; parasitism 38·4 per cent.

Plot III: 202 flies, 272 parasites; parasitism 57·3 per cent.

It will be evident, therefore, that these two years' results agree very closely with those for 1926 and 1927 (Imms, 1930, p. 12.) With regard to the 1930 results, they confirm the previous conclusion that the parasitisation of frit-fly, affecting late sown oats, is markedly heavier than that suffered by the same generation of the insect attacking oats sown earlier in the season. The details concerning the parasites may be summarised as follows.

During 1929, when one field plot was utilised, 26·4 of the individual insects reared were parasites which consisted of the following species: *Halticoptera fuscicornis* 4 ♂♂, 14 ♀♀; *Rhoptromeris eucera* 133 ♂♂, 75 ♀♀; *Loxotropa tritoma* 112 ♂♂, 84 ♀♀; *Callitula bicolor*, 15 ♂♂, 9 ♀♀; *Eucoila* sp. indet. 1 ♀.

During 1930, when three plots were laid down, the rearings of parasites were as follows:

Plot I: *Halticoptera fuscicornis* 14 ♂♂, 18 ♀♀; *Rhoptromeris eucera* 14 ♂♂, 22 ♀♀; *Loxotropa tritoma* 77 ♂♂, 72 ♀♀.

Plot II: *Halticoptera fuscicornis* 44 ♂♂, 54 ♀♀; *Rhoptromeris eucera* 49 ♂♂, 68 ♀♀; *Loxotropa tritoma* 161 ♂♂, 63 ♀♀; *Callitula bicolor* 17 ♂♂, 13 ♀♀; *Eucoila* sp. indet. 2 ♀♀; *Halticoptera* sp. indet. 1 ♀.

Plot III: *Halticoptera fuscicornis* 22 ♂♂, 32 ♀♀; *Rhoptromeris eucera* 24 ♂♂, 32 ♀♀; *Loxotropa tritoma* 76 ♂♂, 34 ♀♀; *Callitula bicolor* 28 ♂♂, 24 ♀♀.

The most noteworthy conclusions to be drawn from these results are that during 1930 the dominant parasite species was the Proctotrypid *Loxotropa tritoma*. *Rhoptromeris eucera*, the dominant parasite during 1926, 1927 and 1929, fell to second place and *Halticoptera fuscicornis* declined to third place. *Callitula bicolor*, a species which was only represented by two examples during 1927, was reared in considerable numbers during 1929 and 1930.

Evidence collected during the years 1926–30 on several hundred host larvae and puparia, each individual being isolated in a separate vial, showed that the imagines of all four of the species of parasites mentioned emerged from frit-fly puparia. Furthermore, with one single exception, in which two males of

Loxotropa tritoma issued from one host puparium, only one parasite emerged from each individual host attacked. It may be taken, therefore, that each individual parasite represents the destruction of one host individual.

Before the actual collection of frit-fly larvae and puparia was undertaken, carefully randomised samples of the plants growing on each of the three 1930 plots were obtained. These were taken up by the roots irrespective of their being infested by frit-fly or not, and they served to ascertain the approximate degree of infestation on each of the plots concerned. The number of plants gathered was neglected and only the actual number of shoots, infested or not, was taken into account. The degree of infestation in each case was determined by the frequency of frit-fly larvae and puparia present in relation to a known number of shoots. The latter, it may be added, included shoots which were healthy, infested or killed, in variable proportions, all being included in the counting. The result of the sampling was as follows:

Plot I: Number of shoots examined 528; number of frit-fly larvae and puparia present 118; percentage of infestation 22·3.

Plot II: Number of shoots examined 1119; number of frit-fly larvae and puparia present 422; percentage of infestation 37·7.

Plot III: Number of shoots examined 961; number of frit-fly larvae and puparia present 289; percentage of infestation 30·1.

While these observations are, by their nature, open to some criticism they appear to show (*a*) the advantage of early sowing of oats in order to reduce infestation by frit-fly; and (*b*) that the high percentage of parasites reared from frit-fly affecting Plot III is due to an increase in the parasite population rather than to an increase in the host population available for parasitisation.

Taken collectively, the observations made between 1926 and 1930 cover a period during which the oat crop in England and Wales suffered low or moderate infestation by frit-fly, and no widespread severe outbreak of this insect was recorded by the Ministry of Agriculture. The percentage of natural parasitisation to which the frit-fly was observed to be subjected may be divided into two classes in direct relation to the sowing date of the oats which the insect infests. (1) In cases where the sowing date for the experimental plots was on or before March 15th the mean percentage of parasitisation was 23. (2) Where the sowing date was after March 15th and up to as late as April 25th the mean percentage of parasitisation was 43·5. It would appear, therefore, that the more intensive parasitisation prevails when the frit-fly affects spring oats sown later than is customary for any average season in England and Wales.

II. On *Callitula bicolor* Spin.

Detailed descriptions of three out of the four species of parasites reared from puparia of the frit-fly have already been given in a previous paper (Imms, 1930), and *Callitula bicolor* alone remains for further consideration. This species is a member of the family Pteromalidae and belongs to the subfamily Merisinae as defined by Ashmead (1904). The tribal division of the Merisini to which

Callitula is relegated includes species that are predominantly parasites of Diptera.

The genus *Callitula* was established by Spinola (1811) in his paper entitled "Classification des Diplolepaires." This latter is a synoptic key to Chalcid genera and contains no complete description of *Callitula* whose only species, viz. *C. bicolor* Spin., is unaccompanied by any description other than the characters upon which its genus was founded. Walker, in 1833, described the insect as a new genus and species, *Micromelus rufomaculatus*. Förster (1856) incorrectly stated that Walker's generic name of *Micromelus* was preoccupied, and substituted the new name of *Baeotomus* in consequence. Two years later Reinhard regarded Spinola's diagnosis as being invalid and adopted Walker's generic and specific names. In Dalla Torre's Catalogue (1898) Förster's nomenclature is accepted, the species being listed as *Baeotomus rufomaculatus* (Walk.). Ashmead (1904) followed Reinhard and accepted *Callitula* as being a synonym of *Micromelus*, but in 1923 Gahan and Fagan pointed out that, according to the rules of nomenclature, the names of both the genus and species, *Callitula bicolor* Spin., are valid and consequently antedate *Micromelus rufomaculatus* Walk.

This insect has been recorded by Marchal, Kurdjumov, Leonardi and others, under the name *Micromelus rufomaculatus*, as a parasite of the Hessian fly (*Mayetiola destructor*) in Europe. Whether it is a primary parasite or a hyperparasite appears to be uncertain, but Kurdjumov (1913) states that he has always found it as a hyperparasite. Its relationship with *Oscinella frit* has not been determined, and it may possibly prove to be a secondary parasite of that species also.

This insect was reared singly from frit-fly puparia between July 18th and 27th, 1929, and between July 19th and August 7th, 1930. I am indebted to Dr Ch. Ferrière of the Imperial Institute of Entomology for confirming my identification. The only previous record of the occurrence of this species in Great Britain is that of Walker (1833) who gives the following: "June: grass in fields: near London. New Lanark, Scotland." Walker's specimens probably parasitised frit-fly affecting wild grasses.

Description of the Female. (Fig. 1.)

Coloration. Head and thorax black with dark metallic green or bluish green reflection. Eyes dark chocolate: ocelli translucent brown. Antennae dark fuscous brown with proximal half of scape testaceous. Mandibles yellow-brown. Legs ochreous yellow to testaceous, fifth joint of tarsi and claws dark fuscous. Tegulae testaceous. Wings hyaline with the veins smoky or smoky yellow. Abdomen black or brown-black, shining, with slight bronzy reflection: and irregular ochreous mid-dorsal area on first and second segments. Ventrally, abdomen is testaceous with the apical half suffused with fuscous.

Head. Facial aspect rather broader than long, as 3 : 2. Eyes moderate-sized, separated on the vertex by a distance equal to the frontal depth of the

head. Ocelli disposed in the form of a flattened triangle, the medium ocellus only slightly in advance of the lateral pair: lateral ocelli separated by an interval rather greater than the distance of either ocellus from the inner margin of the compound eye of its side. Toruli situated near the middle of the facial aspect of the head and separated by a distance rather more than their individual diameter. A rather deep broad antennal fovea extends upwards from just behind the toruli. Clypeus narrow, transverse, its lateral angles rounded and separated by a wide but shallow sinus. Surface of head sculptured with a reticular pattern of hexagonal or pentagonal cells: towards the clypeus the cells become elongated and ultimately become converted into ridges which converge around the clypeus.

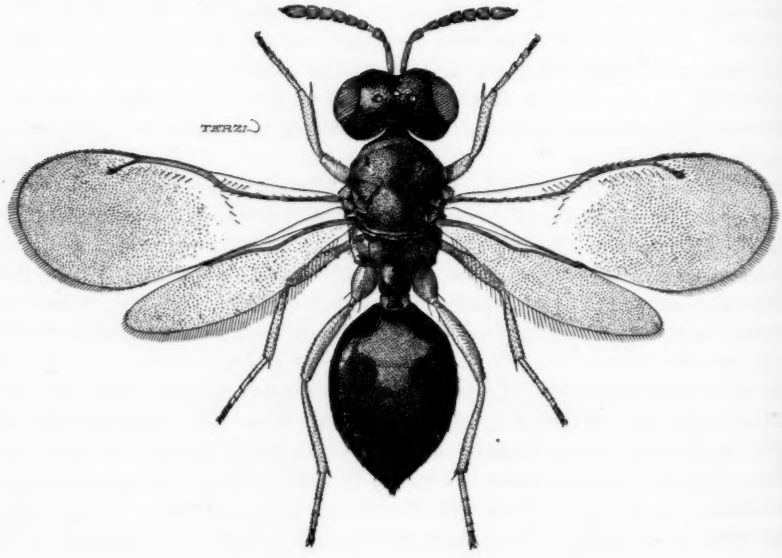

Fig. 1. *Callitula bicolor* Spin., female. ×40.

Antennae. (Fig. 2 A.) Eleven-jointed. Length 0·6 mm.: scape 0·2 mm. long (including small basal sclerite) and 0·02 mm. broad, related in length to pedicel as 9 : 2. Pedicel longer than any of the funicular joints. Ring-joints three, collectively slightly shorter than first funicular joint: joints 1 and 2 together equal in length to 3. Funicular joints subequal in length, diameter of successive joints increasing somewhat. Club half the length of scape—drawn out apically into a rather slender process. Sensoria six to eight on each funicular joint and three whorls on the club.

Mouth-parts. Mandibles (Fig. 2 B, C) heterodont: right 0·16 mm. long and 0·09 mm. broad, with four teeth; left very slightly smaller and with three

teeth. Maxillary palpi four-jointed: joints related in length as 7 : 6 : 5 : 10. Labial palpi three-jointed, second joint minute and obscure.

Thorax. (Fig. 1.) Dorsal and lateral surfaces with a reticulate pattern very similar to that of head and coarsest on mesonotum. Prescutal[1] furrows (notauli) very indistinct and extending only a short distance backwards: scarcely evident in some examples. Scutellum markedly convex from side to side. Metanotum in form of shining upraised transverse band. Propodaeum (Fig. 2 D) sculptured with a finer cellular pattern than on thorax: two longitudinal carinar on either side, innermost pair the better developed: spiracles ovoid.

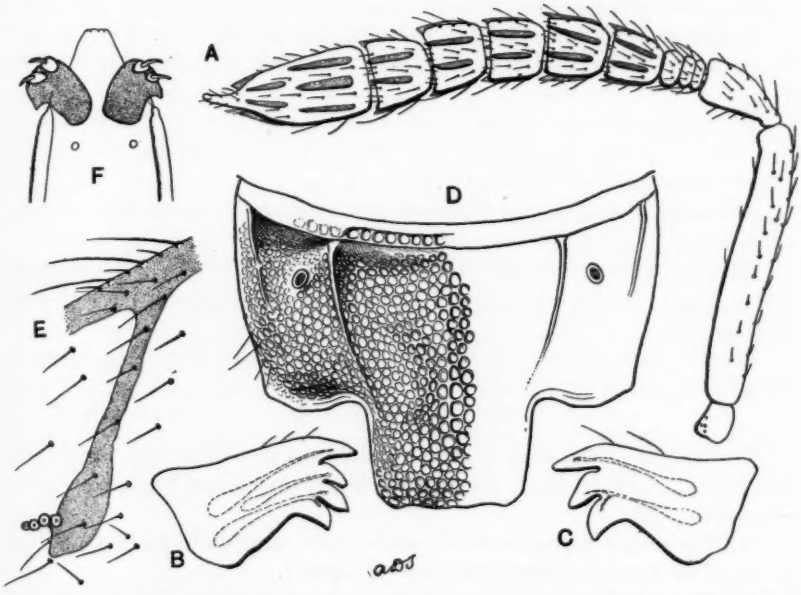

Fig. 2. *Callitula bicolor*, structural characters: with the exception of F, the parts are those of the female. A, left antenna, × 70. B, right mandible, × 182. C, left mandible, × 182. D, propodaeum, × 328. E, stigmal vein and adjacent portion of costal area of left fore-wing, × 350. F, male genitalia, × 130.

Fore-wing. (Fig. 1). With distal three-fifths clothed with fine hairs, basal two-fifths hairless but crossed by an oblique row of four macrochaetae which extends downwards from near the bend of the submarginal vein. Average dimensions of wing (including marginal fringes), length 1·25 mm., breadth 0·55 mm. Submarginal, marginal, postmarginal and stigmal veins related respectively in length as 47 : 25 : 17 : 11. Submarginal vein with twelve macrochaetae: costal cell with a distal row of five or six macrochaetae, re-

[1] Parapsidal furrows of most authorities. For the use of the terms see G. S. Tullock (1929), *Psyche*, **36**, 376.

mainder of cell hairless. Marginal vein with a row of nine or ten macrochaetae along edge of wing: on the wing membrane is a row of nine to eleven macrochaetae near the marginal vein and parallel to it. Stigmal vein with a chain of four annuli (Fig. 2 D).

Hind-wing. Average dimensions (including marginal fringes), length 1·05 mm., breadth 0·29 mm.: marginal vein related to length of wing as 5 : 8.

Legs. Fore-leg with coxa moderately swollen, its length related to breadth as 2 : 1. Femur about equal in length to tibia, its length related to breadth as 13 : 3. Tibia with a spur 0·05 mm. long and unequally bifid at the apex. Tarsal joints[1] related in length as 10 : 7 : 5 : 4 : 7.

Middle-leg with femur related in length to tibia as 6 : 11. Tibia with a slender seta-like spur 0·06 mm. long. Tarsal joints[1] related in length as 13 : 10 : 7 : 5 : 9.

Hind-leg with length of coxa related to its breadth as 2 : 1. Femur slender, only slightly shorter than tibia (7 : 8). Tibia with a spur 0·05 mm. long. Tarsal joints related in length as 15 : 11 : 8 : 6 : 9.

Abdomen. Pedicel with a reticulate pattern dorsally: constricted laterally so as to appear somewhat hour-glass shaped.

Gaster elongate ovoid: first and second terga subequal in length. Spiracles rather large, circular. Apex of ovipositor slightly projecting but not conspicuous.

Length (six examples) 0·95 mm. to 1·25 mm., expanse of wings 2·4 mm. to 3·2 mm.

Description of the male.

The male is similar to or slightly smaller than the female and, apart from the genitalia (Fig. 2 F), it may be readily distinguished by the following characters.

(1) The terminal process on the club of the antenna is wanting and the joints of the funiculus are slightly more elongated.

(2) The dorsal testaceous area on the abdomen is larger and extends over the greater part of the first and second terga.

(3) The ventral fuscous area on the abdomen is more reduced in extent and occupies only the apical one-fourth of that region.

III. General conclusions.

As the result of observations carried out at Harpenden during the years 1926–30, the following conclusions are drawn.

1. The extent of destruction of the stem generation of *Oscinella frit* through the agency of parasites increases progressively with the normal advance of the season. When the host insect infected oats that were sown between February 28th and March 15th the mean percentage destroyed by parasites was 23. When

[1] Measured along the dorsal border.

the sowing dates of oats were between March 20th and April 25th the mean percentage of host insects destroyed by parasites was 43·5. The increase in intensity of parasitisation appears to be correlated with an annual seasonal increase in the parasite population.

2. Three species of primary parasites were involved, viz. the Cynipid *Rhoptromeris eucera* Htg., the Proctotrypid *Loxotropa tritoma* Thoms. and the Chalcid *Halticoptera fuscicornis* (Walk.). A fourth species of parasite, viz. the Chalcid *Callitula bicolor* Spin. occurred in smaller numbers and its status was undetermined. The possibility of its being a secondary parasite requires investigation.

3. The species *Callitula bicolor* is recorded for the first time as a parasite of *Oscinella frit* and a detailed description of the insect is given.

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CORRECTION.

NOTE ON A MICROCOTYLID FROM *SCIAENA ANTARCTICA*.

By FLORENCE V. MURRAY.

In a recent paper (*Parasitology*, **23**, No. 4, 492) I described under the name *Microcotyle sciaenae* a new species of *Microcotyle* from the gills of *Sciaena antarctica*. Dr M. C. Hall, Chief of the Bureau of Animal Industry, Washington, D.C., has kindly pointed out to me that this name is preoccupied by *M. sciaenae* Goto (1894). I now propose the name *M. sciaenicola* in place of this homonym.

(*MS. received for publication* May, 1932.—Ed.)